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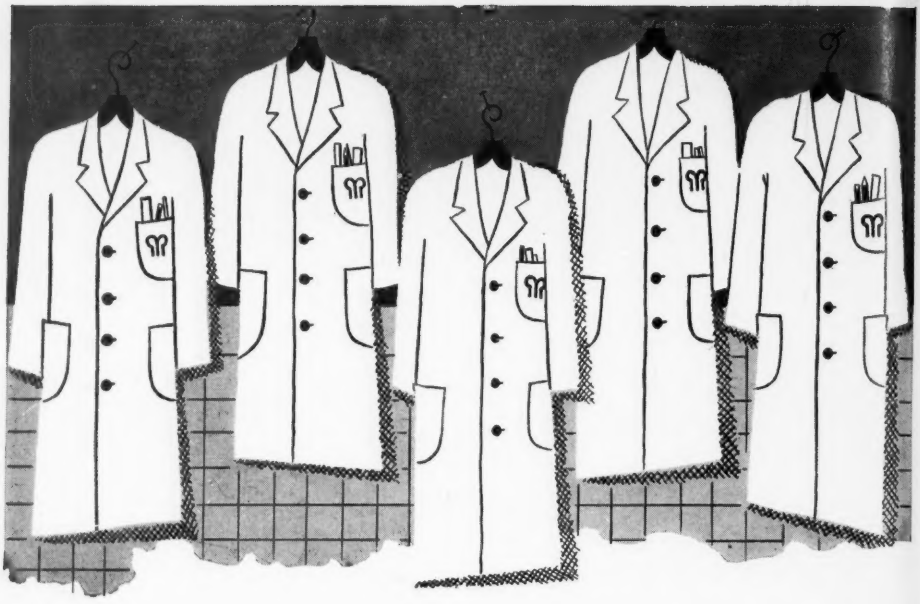

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ADRENAL CORTICAL FUNCTION IN NEWLY-BORN INFANTS

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THE anatomic features of the adrenal cortex of the newborn infant have long been known to differ strikingly from those of the adult. Furthermore, marked involutionary changes occur in this gland during the first few days of extra-uterine life. These facts raise the question as to whether there may be differences in function as well. Efforts to study this problem by assaying the excretion of adrenal cortical metabolites in the urine of newly-born infants have recently been made. These have demonstrated that small amounts of such substances, as measured by both chemical and biologic methods, are present in the urine during the newborn period (1, 2, 3). It has also been shown that the adrenal cortex of the newborn infant responds to stress in a manner similar to that of the adult (3).

The purpose of this investigation was to study this problem further. In order to obtain a more dynamic view of adrenal cortical function in the newborn period, repeated assays of urinary corticoids were made on each infant. In addition, the response of the adrenal cortex to the administration of ACTH was studied.

MATERIALS AND METHODS

This study was carried out on 10 normal male infants delivered at the

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Royal Victoria Montreal Maternity Hospital. All of the mothers had had uneventful pregnancies. The gestation periods were of normal duration with the exception of one infant, BRA, who was born after nine lunar months and had the physical characteristics of a premature baby.

These infants were admitted shortly after birth to the "Premature Nursery" where the environmental conditions were relatively constant. They were immediately given 100 mg. of ascorbic acid and 20 mg. of folic acid intramuscularly. Feeding of clear fluids was begun as soon as the infants were free from mucus and their sucking reflexes well established. The babies were fed cow's milk formula starting on the second day of life, to which was added 25 mg. of ascorbic acid.

Complete urine collections from the time of admission to the Nursery were obtained by means of a funnel-shaped glass tube fastened over the penis. Urine voided was delivered by gravity through a latex tube to a clean glass flask. This flask was emptied at frequent intervals into a bottle and kept in 24-hour amounts in a refrigerator. No preservative was used.

Two lots of porcine adrenocorticotropin were used (Armour's 37-KE and H-2403). The hormone preparation was administered intramuscularly as a solution in distilled water, containing the equivalent of 1.2 to 18 mg. of Armour's Standard per ml., depending upon the desired dosage.

The ACTH was administered at the beginning of a 24-hour urine collection period, and when multiple injections were given, at 6-hour intervals thereafter.

The urinary corticoids were assayed by the method of Daughaday *et al.* (4), which measures the amount of formaldehyde liberated following oxidation with periodic acid. The 17-ketosteroids were determined by the procedure of Callow *et al.* (5), the alcoholic potassium hydroxide being prepared by the method of Wilson and Carter (6). A color correction was applied.

Uric acid was determined by the method of Shaffer (7), which was modified and adapted to Folin's direct method (8, 9) and to the use of an Evelyn photoelectric colorimeter. Urinary creatinine was determined by the method of Folin (10).

The eosinophil counts were made on capillary blood, using the phloxine-propylene glycol diluent described by Randolph (11). The pipettes were shaken in a mechanical shaker for three minutes. A Levy Fuchs-Rosenthal counting chamber was used and the average of two to four counts computed. The absolute eosinophil count was determined by dividing the average chamber count by 16 and multiplying by 100.

The surface area of each infant was determined by the formula of Faber and Melcher (12).

TABLE 1

Name	Surface area	Days of age	ACTH administered	Fluid intake	Urine volume	Creatinine in urine	Uric acid in urine	Glycosuria	Urinary corticoids (oxidation method)	
									Total	Per sq. m
	sq.m.		mg.	ml./24 hrs.	ml./24 hrs.	Gm./24 hrs.	mg./24 hrs.		mg./24 hrs.	mg./24 hrs.
1. Lab.	0.1796	6		375	245	0.13	—		0.112	0.625
		7	0.3	345	304	0.17	—		0.143	0.796
		8		450	275	0.26	—		0.040	0.223
2. Des.	0.2304	8		510	112	0.30	43.5		0.129	0.559
		9	1.5	345	187	0.56	73.0		.172	0.745
		10		645	196	0.49	68.6		0.084	0.363
3. Tem.	0.2043	1		480	84	0.11	—		0.058	0.283
		2		360	106	0.20	—		0.097	0.474
		3		467	190	0.35	—		0.113	0.552
		—		—	—	—	—		—	—
		9		600	290	0.15	71.2		0.119	0.582
		10	2.4	600	280	0.27	83.7		0.125	0.612
		11		780	455	0.33	54.6		0.081	0.397
4. Bras.	0.1643	1		180	4	0.20	—		0.045	0.274
		2		217	39	0.14	—		0.039	0.238
		3		290	64	0.21	—		0.051	0.310
		—		—	—	—	—		—	—
		8		405	50	0.20	17.1	0	0.044	0.267
		9		360	—	—	—	—	—	—
		10	2.8	—	46	0.64	16.4	0	0.071	0.433
		11		700	133	0.66	26.0	0	0.084	0.511
5. Gil.	0.2287	1		225	66	0.42	—		0.082	0.359
		2		300	54	0.32	—		0.067	0.293
		3		486	107	0.20	—		0.074	0.323
		—		—	—	—	—		—	—
		9		668	304	0.42	78.8	0	0.105	0.459
		10	4.8	585	180	0.34	37.1	0	0.068	0.298
		11		720	375	0.46	91.0	0	0.133	0.580
6. Bac.	0.2126	1		225	78	0.38			0.075	0.349
		2		390	36	0.36			0.075	0.349
		3		420	134	9.48			0.114	0.530
		—		—	—	—			—	—
		9		540	280	0.30	64.5		—	—
		10	5.0	488	202	0.52	82.0	—	—	—
		11		630	240	0.43	73.0		—	—
7. Hil.	0.2126	1			98	0.24			0.025	
		2			88	0.31			0.060	
		3			218	0.43			0.066	
8. Bel.	0.2226	1	—	510	172	0.34	106.2		0.042	0.188
		2	20.0*	405	90	0.43	100.5		0.259	1.165
		3		660	240	0.33	78.0		0.143	0.643
		—	—	—	—	—	—		—	—
		8	—	765	374	0.30	85.6		0.067	0.301
		9	20.0*	570	306	0.35	52.5		0.332	1.491
		10	—	840	395	0.39	95.0		0.115	0.516
9. Mil.	0.1906	1	—	300	148	0.17	79.0		0.050	0.262
		2	14.0**	360	146	0.34	90.5		0.130	0.682
		3	—	420	285	0.14	56.5		0.044	0.231
		—	—	—	—	—	—		—	—
	0.2073	8	—	705	354	0.20	62.6		0.091	0.440
		9	14.0**	510	388	0.19	81.1		0.468	2.260
		10	—	630	258	0.23	65.8		0.076	0.376
10. Thi.	0.2543	1	—	270	140	0.45	141.1		0.075	0.292
		2	18.0†	465	104	0.57	117.0		0.081	0.315
		3	—	585	475	0.42	68.0		0.083	0.323
		4	9.0	780	455	0.37	76.0		0.188	0.731
		5	—	720	594	0.29	63.3		0.060	0.225
		6	6.0	720	478	0.50	54.8		0.090	0.350
	0.2595	7	—	720	340§	0.51	42.9		0.059	0.230
		8	5.0	720	480	0.52	83.0		0.173	0.670
		9	—	720	474	0.52	72.4		0.088	0.319
		10	3.0	780	385	0.55	64.9		0.099	0.386

* 5.0 mg. Q 6H×4.
** 7.0 mg. Q 6H×2.
† 9.0 mg. Q 6H×2.
§ One voiding lost—estimated volume 20 ml.

RESULTS

The data obtained in this investigation are reported in detail in Table 1. For purposes of greater clarity, certain of the results are also presented graphically in figures.

Spontaneous excretion

A. Corticoids

Figure 1 presents the assays by the oxidation method obtained in the study of these infants. The total amount of corticoids found in the urine per twenty-four hours was small; the lowest value was 0.025 mg. and the highest was 0.133 mg. The assays obtained in the first three days of life, and particularly in the first two, tended to be lower than those in the sec-

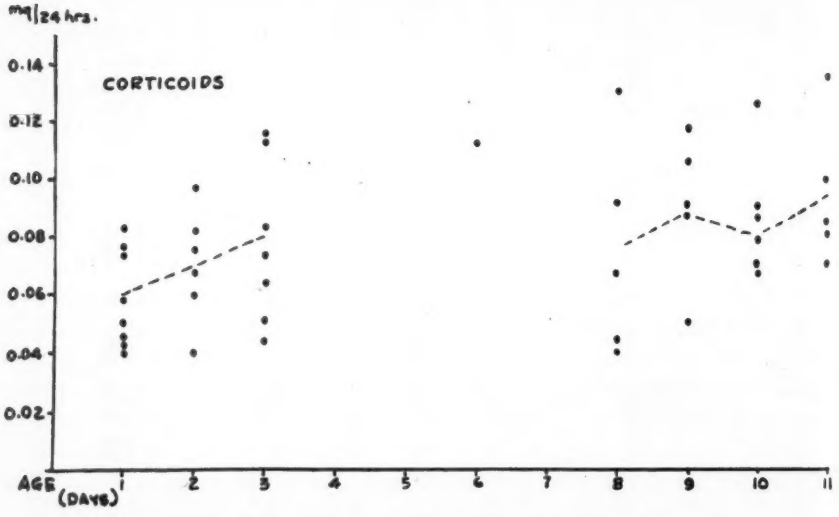

FIG. 1. Excretion of formaldehydogenic corticoids in newly-born infants.

ond week. When the average values obtained in the first two postnatal days were compared statistically with those found in the second week, the differences were highly significant.

B. 17-Ketosteroids

It was found that the amount of 17-ketosteroids present in the urine of the first baby in which they were determined (THI) was considerably greater in the first few days of life than in the second week. For instance, in the twenty-four hours following birth, this baby excreted 1.75 mg.,

whereas on the ninth day the assay value was 0.75 mg. This observation, since it was on a baby which had received ACTH, was not necessarily typical of newly-born infants, so that 3 additional normal male infants were studied. Figure 2 presents the data obtained. The 17-ketosteroid assays were high in the first two days of life, in 2 of the infants exceeding 1.5 mg. per twenty-four hours; thereafter the amounts excreted decreased rapidly. On the eighth day the assays were less than one third of those found on the second day. The third infant showed little change in the 17-ketosteroid excretion, the values throughout the experimental period being approximately the same as found in the other infants on the eighth day.

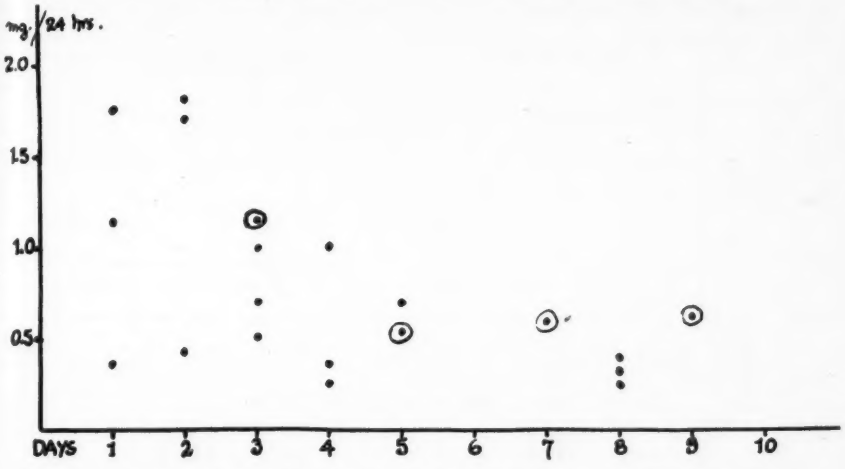

FIG. 2. Excretion of 17-ketosteroids in newly-born infants. Encircled dots represent values obtained on Baby Thi., who received ACTH on the previous days.

The effect of ACTH

All of the infants became pale immediately following the injection of ACTH except the 2 receiving the smallest doses. This pallor lasted about three hours. A local reaction was observed once, which consisted of a raised pale area about 3 cm. in diameter at the site of injection. It subsided in about half an hour.

A. Corticoids

The first 5 infants studied were given ACTH as a single injection on the seventh, ninth or tenth days of life. Each baby received a larger amount of the hormone than the preceding one. The largest single dose administered to this group was 4.8 mg. The data in Table 1 show that no increase in the excretion of corticoids resulted.

The next infant (BEL) was given 4 injections of ACTH on both the second and ninth days of life. These injections were at 6-hourly intervals and each contained 5 mg. (22.4 mg. per sq. m.) of ACTH. The results are also presented graphically in Figure 3. There was a significant rise in the excretion of corticoids, the response being somewhat greater on the ninth day than on the second.

A marked rise in corticoid excretion also was observed following the administration of ACTH divided into two doses (MIL). This time 7 mg.

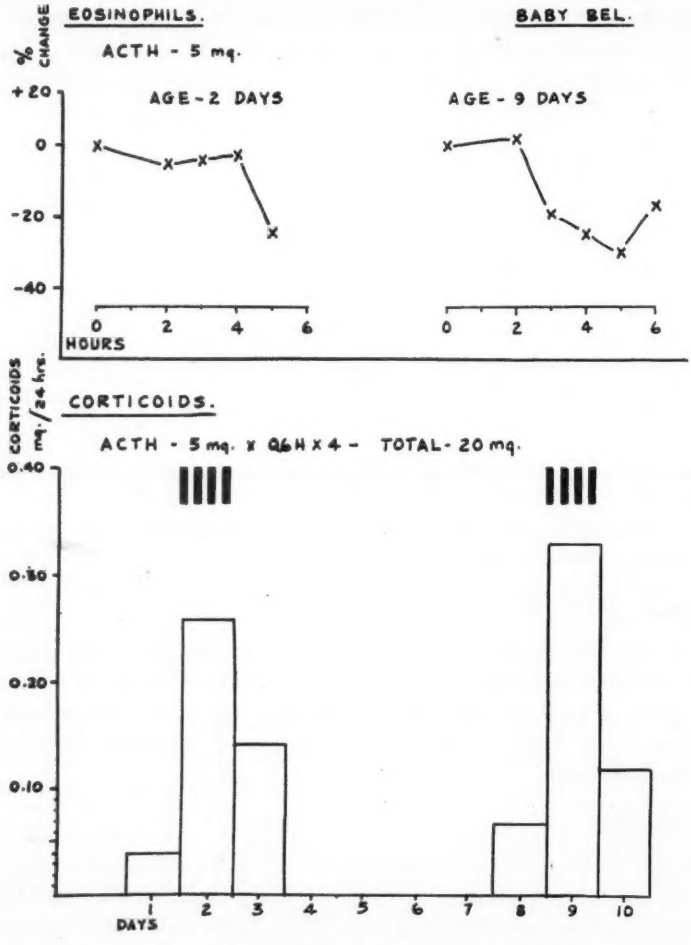

FIG. 3. Effect of ACTH on the excretion of corticoids and eosinophil counts in infants.

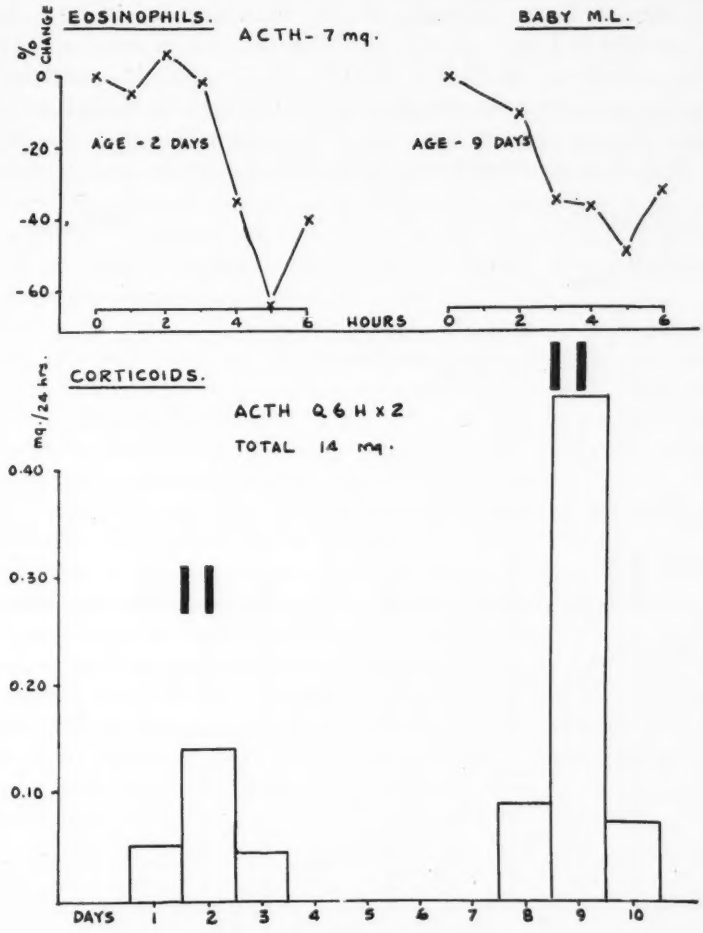

FIG. 4. Effect of ACTH on the excretion of corticoids and eosinophil counts in infants.

(36 mg. per sq. m.) was given at the beginning of the urine collection day and repeated six hours later. The results, shown in Figure 4, indicate that the response was much greater on the ninth day than it was on the second.

In Figure 5 the results obtained from the administration of ACTH every other day are depicted (THI). On the second day of life, the baby received a total of 18 mg. (70 mg. per sq. m.) in two doses separated by six hours. In contrast to the effect observed in the preceding experiment, there

was no increase in the excretion of corticoids. Forty-eight hours later a single injection of 9 mg. resulted in a rise of the urinary corticoids. Six mg. (23.3 mg. per sq. m.) of ACTH caused a slight rise on the sixth day, but this was not as great as the response which followed the injection of 5 mg. (19.4 mg. per sq. m.) two days later. The last injection of the hormone which this infant received was 3.0 mg. (11.7 mg. per sq. m.) when he was

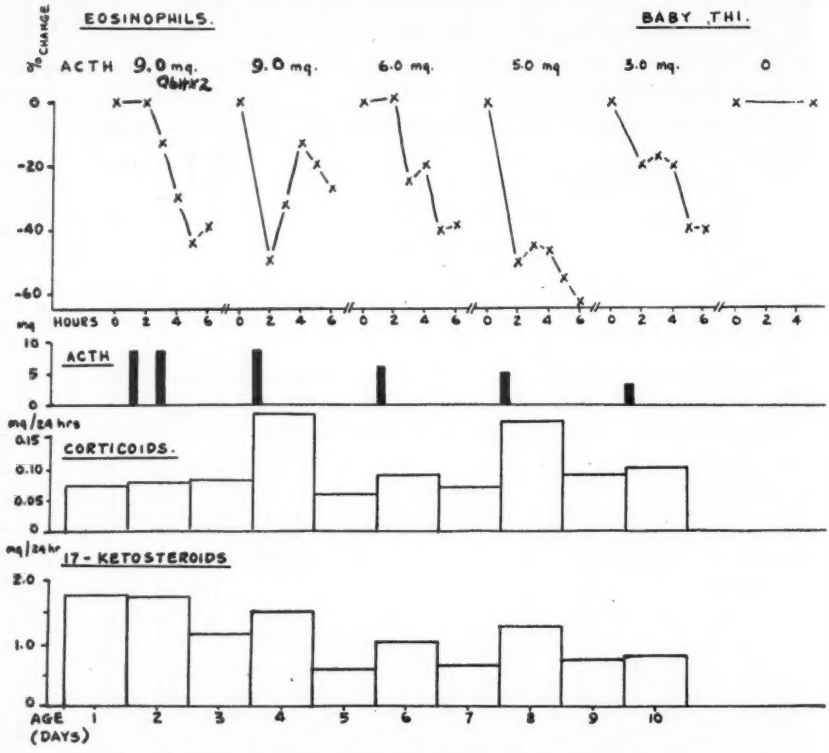

FIG. 5. Effect of repeated administration of ACTH on the excretion of corticoids, 17-ketosteroids and eosinophil counts.

10 days old. This resulted in a very questionable increase in the amount of corticoids excreted. On only the fourth and eighth days did the total amount of urinary corticoids exceed the normal range.

B. 17-Ketosteroids

The urinary 17-ketosteroids were measured in the infant that received ACTH on alternate days. The results are shown in Figure 5. As was noted in the corticoid assays, the most marked responses occurred on the

fourth and eighth days, with a lesser rise being observed on the sixth day of life.

Eosinophils

Due to technical difficulties prior to the adoption of Randolph's phloxine-propylene glycol diluent, satisfactory eosinophil counts were obtained only on the last 4 babies which received ACTH. The eosinophil counts were made immediately prior to the injection of ACTH and at hourly intervals for six hours thereafter. The counts made before the hormone was given varied from 468 to 969 eosinophils per cu. mm. of blood and are normal values for this age group (13).

The injection of ACTH was invariably followed by a decrease in the number of blood eosinophils in these 4 babies (Figs. 4, 5, 6,) the maximum decrease usually occurring at the fifth hour. This was observed even when there was no increase in the excretion of urinary corticoids. Furthermore, as is shown in Figure 5, the greatest percentage change in eosinophils did not always coincide with greatest rise in corticoids.

Urinary uric acid, creatinine and glucose

The urinary uric acid, and creatinine data are presented in Table 1. The creatinine values are notable for their wide daily variations, even in the same infant, as well as for a lack of correlation between the size of the infant and the amount of creatinine excreted. The uric acid excretion was also erratic, with an irregular downward trend with increasing age being apparent in the few determinations made. No consistent correlation between an increase in the excretion of uric acid and the uric acid-creatinine ratio was found. This contrasts with the changes in this ratio observed in adults (14).

DISCUSSION

Determined by the oxidation method, the amounts of corticoids excreted in the urine of newly-born infants are small. These findings agree with those of Matson and Longwell (2), who measured the urinary lipid-soluble substances, and of Venning *et al.* (3), who measured the urinary glycogenic corticoids. The excretion of such small amounts of corticoids invites the comparison of them with the amounts excreted by the more mature organism. Such a comparison may be based on either body weight or surface area. Matson and Longwell (2), using the former basis, found that per kilogram of body weight the amount of lipid-soluble substances excreted in each twenty-four hours by the newly-born infant was somewhat greater than the amount excreted by the adult. When the values obtained for formaldehydogenic corticoids in the present investigation are

compared with those of adults (15), again the amount per kilogram of body weight is slightly higher in the infant. Talbot (16) has stated that the urinary excretion of nonketonic reducing corticoids is of the same order of magnitude in children and adults when expressed in proportion to surface area. On this basis, the excretion of urinary corticoids by the oxidation method varied from 0.117 to 0.582 mg. per sq. m. per twenty-four hours, compared to the adult male range of 0.292 to 0.490 mg. per sq. m. per twenty-four hours (15). However, those assays that were significantly lower than the minimum adult values were in the first two days of life. It is therefore clear that, with the possible exception of these two days, the excretion of adrenal cortical metabolites by newly-born infants compares favorably with that of the adult.

The studies of Lewis and Pappenheimer (17), Benner (18), and Swinyard (19) have established that the large size of the adrenal at birth is due to a specially developed zone of cells lying in the area later occupied by the adult reticular zone. Furthermore, it has been shown that this zone undergoes involution beginning at or just prior to term, at first slowly, but thereafter very rapidly, resulting in a loss of 50 per cent in the total gland volume during the first two weeks (19). Venning *et al.* (3) have recently pointed out that their findings of a gradual increase in the excretion of glycogenic corticoids at the time this involution is occurring do not support the suggestion that this zone is elaborating these hormones. Additional evidence favoring their conclusions is the fact that the formaldehydogenic corticoids follow a similar pattern. A more probable explanation is that the corticoids are produced by that part of the fetal cortex which develops into the adult cortex. This zone is relatively undifferentiated at birth, the adult-type arrangement first appearing during the second week (19).

The source of the 17-ketosteroids found in the urine of newly-born infants is unknown. They may be maternal in origin, reaching the baby by passing the "placental barrier." Supporting this thesis is the rapid decrease in the amounts excreted with advancing age. On the other hand, they may be in part or entirely of adrenal origin. It is generally agreed that the normal adult cortex produces substances which are excreted in the urine as 17-ketosteroids. Since the administration of ACTH resulted in a rise of urinary 17-ketosteroids, it is evident that the newborn's adrenal cortex can also produce these steroids. That neither Carnes (20) nor Gersh and Grollman (21) were able to find any androgenic activity in extracts of the human fetal adrenal cortex does not negate this view, for the adrenal cortical 17-ketosteroid precursors may not be androgenic. As Talbot (16) has recently pointed out, the precursors of the 17-ketosteroids found in normal adult females are apparently not androgenic, since they have not

caused clitoral hypertrophy or other evidence of masculinization. They are responsible only for pubic and axillary hair growth. Possibly the fetal reticular zone is the source of these 17-ketosteroid precursors but elucidation of this must await further study.

The increased corticoid excretion resulting from the administration of ACTH clearly demonstrates that the adrenal cortex of the newly-born infant is capable of responding to stimulation. This fact has been demonstrated previously by the increased amount of glycogenic corticoids in the urine of 2 infants suffering from atelectasis and studied in this clinic (3). However, the data obtained in this investigation also showed that the magnitude of the response to ACTH was greater in the second week of life. As these infants had received an earlier injection of ACTH, the greater response on the second administration might have been due to a 'priming' of the adrenal. In another infant, a response was observed with half the dose which two days previously had shown no effect. It is not possible, from these studies, to decide whether this increased responsiveness is caused by 'priming' of the adrenal with ACTH or whether it may be attributed to the natural increase in maturity of the adrenal in the older infants.

It should be emphasized, however, that the failure to detect an increase of urinary corticoids after the administration of ACTH does not mean that the adrenal cortex has not responded. This is well demonstrated by the fact that the blood eosinophils decreased even when there was no rise in corticoid excretion. It may be that the eosinophils are a more sensitive index of adrenal cortical activity than changes in amounts of corticoids excreted in the urine. On the other hand, when the corticoids did increase, the eosinophils did not change in like proportion, thus indicating that these two indices of adrenal cortical activity are to some extent independent of each other.

One of the most sensitive criteria for determining increased adrenal cortical activity in the adult is an increase in the uric acid: creatinine ratio (14). This ratio apparently cannot be used an an index of such activity in newly-born infants. It is generally believed that the metabolism of uric acid and of creatinine in neonatal life differs from their metabolism in older age groups; thus, whereas the excretion of creatinine in the adult is related to the active muscle mass and is therefore relatively constant in any one individual, creatinine metabolism in the newly-born infant is not entirely independent of other elements of nitrogen metabolism (22). The wide daily variations in creatinine values as observed in these babies is consistent with this view.

The various factors affecting the metabolism of purine nitrogen in the newborn also are not well understood (23), nor has the subject been reexamined with modern methods. The range of urinary uric acid values ob-

tained in these babies was considerably lower than reported in newly-born infants by Schloss and Crawford (24).

Although the possible influence of deficient renal and hepatic functions cannot be disregarded, the data obtained in this and previous studies indicate a degree of functional immaturity of the adrenal cortex in the early neonatal period.

SUMMARY

The 24-hour urinary excretion of corticoids in 10 newly-born infants 1 to 11 days of age was determined serially, using the oxidation method. Small amounts of corticoids were found. There was a definite trend towards increasing excretion of these substances with increasing age, the difference between the amounts present in the urine in the first two days of life and in the second week of life being highly significant. Per square meter of body surface, the amounts of corticoids excreted during the second week of life were of the same order of magnitude as those excreted by normal adult males.

The amounts of 17-ketosteroids excreted by 1 of these infants and 3 additional infants were also determined. Relatively large amounts were found in the first two days of life, followed by a definite downward trend, the values by the ninth day being less than one third of those found originally.

Varying amounts of ACTH were administered intramuscularly. When a sufficient amount of this hormone was given, the infants responded by a rise in the urinary excretion of corticoids, a rise in urinary 17-ketosteroids and a fall in blood eosinophils. The uric acid : creatinine ratio did not prove to be a satisfactory index of the adrenal cortical activity in this age group. The responsiveness of the adrenal cortex to ACTH was found to be increased in the second week of life.

Acknowledgments

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METABOLIC CONSEQUENCES OF SPINAL CORD INJURY

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THE clinical course of many patients after severe trauma to the spinal cord is often marked by the inability to ingest adequate amounts of protein and the failure to assimilate fully protein which is ingested or administered parenterally (1, 2). It is not a rare occurrence to see a patient who is paraplegic as the result of injury to the spinal cord, literally "die of starvation," despite vigorous attempts to supply adequate nutrients (Case 6). Our study was undertaken to gain further factual insight into the pathogenesis of the derangement of protein metabolism in such cases. In addition we have recorded clinical observations of gynecomastia, testicular atrophy and other endocrinologic variations which have been reported in cases of paraplegic men (3, 4).

MATERIALS AND METHODS

Clinical and metabolic studies were made of the cases of 16 men who had suffered injury to the spinal cord. The investigation was limited to hospitalized patients, who, for the purpose of this report, were divided into 3 groups, depending on the interval which elapsed from the time of injury until the time the essential data were recorded. These arbitrary groups included cases in which the condition of the patient was 1) acute (recent), being observed during the first two weeks after injury, 2) postacute, with examination from two weeks to six months after injury and 3) chronic, observed six months or longer after injury. Four patients are considered in more than one of these categories.

Studies of controlled metabolic balance could not be carried out, inasmuch as all patients were incontinent. There was loss of protein-laden exudate from decubitus ulcers in certain cases. It was understandably necessary to vary the intake of food according to the patient's ability to eat. Other complications were common such as genito-urinary infections and urinary calculi. In spite of these difficulties, every effort was made to

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control as many factors as possible. The results cannot be quantitatively accurate, but they do indicate metabolic trends in the responses of previously healthy men to injury of the spinal cord.

EFFECT OF TRAUMA FROM THE STANDPOINT OF THE LITERATURE

On catabolism.—Cuthbertson (5) called attention to the catabolic effect of trauma on the protein stores in the body and demonstrated that forced feeding of high protein and high caloric diets failed to prevent a negative nitrogen balance at the height of the catabolic process. This is in marked contrast to the result of overfeeding normal uninjured persons, in whom excess food will result in marked retention of nitrogen (6). Breakdown of muscle has been said to account for four fifths of the loss in weight after trauma, the remainder being due to oxidation of carbohydrate and reserve fat (7).

Elevation of nonprotein nitrogen in the urine has been reported after burns (8) and subsequent to operation (9). In each of these instances the amount of nitrogen lost was proportional to the intensity of the burn or surgical procedure respectively.

Howard (10) summarized the known facts concerning the post-traumatic destruction of protein in the body as follows: 1. Loss of nitrogen is brought about by increased excretion of nitrogen in the urine, there being no more than a normal amount of nitrogen in the stools; hence, the reaction does not involve an abnormality in the intestinal absorptive mechanism. 2. The nitrogenous products of catabolized protein appear in the urine in the same pattern as in health, that is, mostly in the form of urea. Excretion of creatine, however, is increased disproportionately to the other components. 3. Fever alone will not produce the reaction. 4. Large quantities of protein, administered orally or parenterally, do not spare body nitrogen at the height of a vigorous catabolic reaction.

Browne and co-workers (11, 12) have demonstrated that debilitated persons do not exhibit a catabolic reaction after trauma. Consequently, the severity of the catabolic reaction depends on the intensity of the traumatic stimulus and on the ability of the organism to respond. The mechanism responsible for the post-traumatic catabolic response has not been elucidated satisfactorily as yet.

It has been established that atrophy which results from disuse will account for only a small fraction of the nitrogen excreted in the urine after trauma (7, 10). Rest in bed and immobilization of healthy young men has resulted in increase in excretion of nitrogen, which is minute compared to the loss of nitrogen during the catabolic period after trauma (13).

On basal metabolism.—Henderson, Prince and Haggard (14) noted an early decrease in basal metabolic rate after burns. Aub (15) observed a fall

of the basal metabolic rate in cats, which was roughly proportional to the severity of shock produced. Selye (16) also reported a lowering of the basal metabolic rate after severe physical stress.

On excretion of hormones.—The excretion of 17-ketosteroids in the urine is said to be increased for twenty-four to forty-eight hours after trauma; it then falls below normal for a period approximating the catabolic reaction (17, 18).

On liver function.—The impairment of liver function after trauma has been reported recently (4, 19, 20), but as yet this has not been widely investigated. The deleterious effect of depletion of protein on the liver must be considered in evaluating the post-traumatic function of this organ (21, 22). Beecher (19), in a study of severely wounded soldiers, found retention of bromsulfalein in 59 cases to be 12.4 ± 1.2 per cent within the first twenty-four hours after injury. He found no correlation between the degree of retention and shock, and this could not be correlated with the degree of blood lost or fluctuations in the volume of blood.

GYNECOMASTIA IN PARAPLEGIC MEN

Cooper and Hoen (3) reported that gynecomastia occurred in 7 of 32 cases of paraplegic men. In the case of one young man gynecomastia, hypoproteinemia, lowered basal metabolic rate, hepatomegaly and "demasculinization" developed after subtotal spinal cordectomy (4). The case of this last patient is included in our series.

PROTEIN METABOLISM AFTER INJURY TO THE SPINAL CORD

The acute period.—Severe injury to the spinal cord was followed by a marked increase in the catabolism of protein in the body, which was reflected in the excretion of large amounts of nitrogen in the urine. In some instances the level of serum protein fell rapidly and the albumin-globulin ratio tended to become reversed.

In some instances the urinary nitrogen amounted to as much as 22 to 29 Gm. each day for two to four weeks after trauma, the peak being reached during the third to sixth days. These levels of excretion of urinary nitrogen were observed despite a limited intake of nitrogen, which varied from 0 to 12 Gm. daily during this period. Parenteral administration of additional amounts of protein produced an increase in the amount of nitrogen excreted. The distribution of nitrogen in the urine was essentially normal, and urea nitrogen formed 80 per cent of the total urinary nitrogen. The creatine nitrogen, however, was increased disproportionately and sometimes amounted to as much as 500 mg. daily. The degree of loss of nitrogen appeared to be directly proportional to the severity of the injury to the spinal cord (Table 1). Concussion of the spinal cord, although productive

of a temporary paraplegia or quadriplegia, initially indistinguishable from that produced by transection of the cord, did not result in a marked catabolic response.

Concentration of protein in the serum decreased within seven to fourteen days after injury to 4.6 to 5.7 Gm. per 100 cc. (normal values 6.4 to 7.6 Gm. per 100 cc.). Although the decrease in serum protein may be modified by the administration of a high protein diet or protein hydrolysates, it has not been prevented by such measures. Reports of 4 representative cases follow:

Case 1.—A carpenter, 68 years of age, fell nine feet from a scaffold, suffering a fracture-dislocation of the cervical spine with immediate loss of function below the fifth cervical segment. He was admitted to the hospital four days after the injury at which time the serum protein was 5.8 Gm. per 100 cc. One week later, despite vigorous attempts to nourish the patient adequately, the serum protein totaled 5 Gm. per 100 cc. During this week the nitrogen excreted in the urine averaged 24 Gm., reaching 29 Gm. on the fifth day after injury. There was a negative nitrogen balance of 20 to 25 Gm. daily during this week (the fifth through twelfth days after injury). Urinary creatine nitrogen averaged 355 mg. daily during the acute period. Although the patient was placed on a Stryker frame on admission and turned at frequent intervals thereafter, a sacral decubitus ulcer developed on the ninth day after injury.

Case 2.—A farmer, 68 years of age, fell from a corn picker and was rendered quadriplegic on May 12, 1948. He was admitted to the clinic eight hours after injury. Twenty-four hours after injury a sacral decubitus ulcer was evident. On the day of his admission the value for serum protein was 6.8 Gm. per 100 cc. One week after injury the value was 5.6 Gm. per 100 cc. It was decreased further to 4.6 Gm. per 100 cc. two weeks subsequent to injury, at which time the albumin-globulin ratio was 1.1. At this time the patient was markedly edematous. Forty-eight hours after injury, despite adequate urinary excretion, the blood urea was 94 mg. per 100 cc., declining to 54 mg. per 100 cc. two weeks after the injury.

Case 3.—A farmer, 46 years of age, suffered immediate paralysis and loss of sensory perception below the seventh cervical dermatome when he fell seven feet from a hayloft, incurring a fracture-dislocation of the sixth cervical vertebra. Sensory perception returned in the entire right upper extremity during the ensuing two hours. During the eight days subsequent to injury the average excretion of urinary nitrogen was 22 Gm. in twenty-four hours, there being a negative nitrogen balance of 15.99 Gm. daily and a daily excretion of 219 to 389 mg. of creatine nitrogen. Three days after injury the concentration of protein in the serum was 6.2 Gm. per 100 cc. with an albumin-globulin ratio of 2.1. On the seventh day the value for serum protein was 5.6 Gm. per 100 cc. with an albumin-globulin ratio of 1.9.

Case 4.—A white man, 21 years of age, suffered a concussion of the cervical portion of the spinal cord when he dived into shallow water June 11, 1949. He was immediately paralyzed below the level of the shoulders. Within three days, sensation of fullness of the urinary bladder returned, followed by complete recovery of motor and sensory functions six weeks after injury. Only a slight catabolic effect was evident. The serum proteins did not fall below 6.0 Gm. per 100 cc., decubitus ulcers did not tend to form and the patient's appetite remained good. One month after injury the serum proteins measured 6.5 Gm. per 100 cc. Of the 5 patients examined during the acute post-traumatic period,

this patient was the only one who had a concussive injury, and the only one who did not demonstrate a marked catabolic reaction.

Further data on the first 4 cases are tabulated in Table 1.

TABLE 1. CORRELATION OF EXCRETION OF URINARY NITROGEN, URINARY CREATINE NITROGEN, AND SERUM PROTEIN WITH SEVERITY OF INJURY TO THE SPINAL CORD

Case	After injury, day	Total urinary nitrogen lost in twenty-four hours, Gm.	Urinary creatine nitrogen excreted in twenty-four hours, mg.	Concentration of serum protein, Gm. per 100 cc.	Albumin-globulin ratio	Severity of injury to spinal cord (graded on degree of eventual recovery)*	Severity of catabolic reaction
1	4	26.7	300	5.8	1.9		
	6	29.5	337			3	4
	9	23.2	427	5.0	1.8		
2	2	Not	Not	6.8	1.5		
	6	obtained	obtained	5.6	1.7	4	4
	9			5.1	1.0		
	14			4.6	1.1		
3	3			6.2	2.1		
	6	21.5	219	5.6	2.2	2	2
	9	21.3	172	5.6	1.9		
4	4	Not	Not	6.6	1.9		
	8	obtained	obtained	6.0	2.0	1	1

* Graded on a basis of 1 to 4.

The postacute period.—The degree of nitrogen wastage and its duration depends on the severity of the initial catabolic reaction and the ability of the individual to recover. The average daily excretion of nitrogen in the urine during the second through the fifth week was 16.5 Gm., while the intake of nitrogen during this period averaged 5 to 12 Gm. in twenty-four hours. If, in addition, protein hydrolysates were administered parenterally, the urinary excretion of nitrogen was increased proportionately. During the ensuing months a normal balance of nitrogen usually developed. Creatine nitrogen was excreted in amounts up to 100 mg. daily throughout the postacute period, sometimes persisting after the balance of nitrogen had been re-established. Although rising above the lowest values recorded, the concentration of protein in the serum usually continued in the subnormal range.

Two patients demonstrated a markedly positive nitrogen balance (4 to 7 Gm. positive balance daily) during the third and fifth months after injury respectively. Despite this positive nitrogen balance the serum protein remained in the hypoproteinemic range.

In one case (Case 6) the initial catabolic reaction failed to reverse and the patient died during the postacute period.

Case 5.—A 40-year-old farmer was paraplegic below the twelfth dorsal segment after he was carried twenty yards in the air during a cyclone and thrown forcibly to the

ground. During the second through the fifth weeks after injury the total daily excretion of urinary nitrogen averaged 16.8 Gm. while that of creatine nitrogen averaged 82 mg. (Intake of nitrogen during this period averaged 7 to 11 Gm. daily.) During the ensuing three weeks the total daily urinary nitrogen and creatine nitrogen averaged 11.76 Gm. and 62 mg. respectively. During this latter period the balance of nitrogen was re-established and on several days a positive balance of 3 to 5 Gm. was present. The serum protein fluctuated between 5.4 and 5.8 Gm. per 100 cc. with an albumin-globulin ratio of 1.7 during the postacute period.

Case 6.—A white man, 44 years of age, suffered transection of the spinal cord in an automobile accident in September, 1948. He remained completely paralyzed below the sixth thoracic myotome until his death. He was admitted to the clinic three months after injury, at which time the serum protein measured 5.6 Gm. per 100 cc. with an albumin-globulin ratio of 1.0. Several large decubitus ulcers were present over the back and hips. Because the patient refused oral feedings, daily parenteral feedings were necessary. He received liberal quantities of whole blood, plasma and protein hydrolysates. Despite these therapeutic efforts, the concentration of serum proteins fell to 5.1 Gm. per 100 cc. four months after injury and to 4.5 Gm. per 100 cc. five months after injury. It reached a low of 4.2 Gm. per 100 cc. six months after injury, at which time the albumin-globulin ratio was 0.68. Macrocytic anemia was present from the fourth month after injury and on April 1, 1949, seven months after the trauma to the spinal cord, the patient died.

The chronic period.—During this period a positive nitrogen balance may not be established in the case of the paraplegic patient, in which event chronic debilitation develops, a state almost invariably complicated by intractable decubitus ulcers. In other instances, in which a positive nitrogen balance develops, gradual improvement occurs to the point where advanced physical rehabilitation can proceed. These alternative courses were evident in Cases 7 and 8.

Case 7.—A 28-year-old male suffered trauma to the spinal cord and resultant paraplegia in an automobile accident in December, 1947. Eleven months after injury, the concentration of protein in the serum was 6.0 Gm. per 100 cc. with an albumin-globulin ratio of 1.0. Decubitus ulcers over both hips and the sacrum responded poorly to treatment, and forced feeding of a diet high in protein and calories failed to elevate the concentration of serum proteins. In April, 1949, sixteen months after injury, a negative nitrogen balance of 1.7 Gm. daily was recorded; hypoproteinemia continued, and persistent decubitus ulcers rendered the application of walking calipers impossible. It was impossible to re-establish an adequate nitrogen balance, and the patient's rehabilitation was indefinitely delayed.

Case 8.—A laborer, 21 years of age, was pinned beneath a falling tree in March, 1949, and suffered a transection of the spinal cord at the tenth thoracic segment. He has remained totally paraplegic below this level to the time of our report. He was admitted to the clinic seven months after injury, at which time the serum proteins measured 6.4 Gm. per 100 cc. with an albumin-globulin ratio of 1.9. Nitrogen excreted in the urine averaged only 7 Gm. daily, including from 30 to 60 mg. of creatine nitrogen. There was a daily positive nitrogen balance of approximately 5.5 Gm. during a period of two weeks of controlled observation. A sacral decubitus ulcer, present on admission, healed after conservative therapy, while 2 decubitus ulcers over the hips healed sufficiently to

make operation possible. During the first six weeks of hospitalization, the patient gained 12 pounds (5.4 Kg.), from 85 to 97 pounds (from 38.6 to 44.0 Kg.), and by that time was receiving advanced physical therapy as a preliminary to walking on crutches.

LIVER FUNCTION AFTER INJURY TO THE SPINAL CORD

The results of the bromsulfalein test were found to be significantly altered in 3 of the 4 cases of acute injury to the spinal cord in our series.

In these 3 cases retention of bromsulfalein varied from grade 2 (18 per cent retention) to grade 4 (40 per cent retention)during the first two weeks after injury. In Case 1, results of the bromsulfalein test returned to normal three weeks after the patient was injured. In case 3, results of the test returned to normal six weeks after the patient's injury. In Case 2 retention of dye, grade 2 (20 per cent), was still present at the time of the patient's death ten weeks after injury.

In only 1 of the remaining cases in our series (patients examined during the postacute and chronic periods) was the result of the bromsulfalein test abnormal. This was in Case 6, in which retention of dye, grade 1 (10 per cent), was present five months after the patient's injury.

The degree of retention of bromsulfalein was not related to loss of blood (absent or negligible in these cases), elevation of temperature (tests were performed only when patients were afebrile) or transfusions of blood. It appeared to be related to the severity of acute injury to the spinal cord and to the consequent catabolic response.

BASAL METABOLIC RATE AFTER INJURY TO THE SPINAL CORD

During the acute post-traumatic period the average basal metabolic rate was minus 10 per cent. The average basal metabolic rate was minus 14 per cent during the postacute period and minus 21 per cent during the chronic period.

HORMONE EXCRETION AFTER INJURY TO THE SPINAL CORD

It is difficult to make generalizations when the series of cases is small. However, it would appear that within two weeks after injury to the spinal cord the urinary excretion of the 17-ketosteroids and corticosteroids is decreased to a low-normal or subnormal range, remaining in this range throughout the duration of the catabolic reaction. The degree of decrease in the urinary excretion of these substances appears to be directly proportional to the severity of the catabolic reaction. In our series, the 4 patients who died had the lowest excretion of 17-ketosteroids, the excretion for a period of twenty-four hours being below 2 mg. in each case (Cases 1, 2, 7 and 9). The urinary excretion of 17-ketosteroids was subnormal in 8 of the 16 cases, low-normal in 5 and in the middle or upper normal range in 3.

Urinary excretion of 17-ketosteroids and corticosteroids for each case is given in Table 2.

In 10 cases the urine was examined for pituitary gonadotropic hormone by the method of Frank and co-workers (23), and in each instance results of the test were negative for normal amounts.

TABLE 2. CORRELATION OF URINARY EXCRETION OF 17-KETOSTEROIDS AND CORTICOSTEROIDS WITH CLINICAL AND LABORATORY DATA

Case	Level of lesion of spinal cord	Time after trauma that tabulated data were recorded	Lowest level of protein recorded, Gm. per 100 cc. of serum	17-Ketosteroids, mg. in 24 hrs. (normal values 6 to 20.8 mg.)	Corticosteroids, mg. in 24 hrs. (normal values .3 to 1.15 mg.)	Basal metabolic rate %	Gynecomastia	Testicular atrophy	Severity of trauma (grade 1 to 4)
1	Cervical	8 wk.	5.0	0	0.15	Too ill to obtain	0	Positive histologically	3*
2	Cervical	3 wk.	4.6	1.9	0.21	−21	0	0	4*
3	Cervical	1 wk.	5.6	6.8	0.54	−11	0	0	2
4	Cervical	1 wk.	6.0	6.3	0.27	− 7	0	0	1
		4 wk.	6.4	7.2	0.54				
5	Thoracic	5 wk.	5.4	5.4	0.21	−12	0	0	2
6	Thoracic	5 mo.	4.2	1.5	0.40	Not obtained	+	+	3+*
7	Thoracic	11 mo.	6.0	2.3	0.30	−22	+	+	2+
8	Thoracic	7 mo.	6.4	6.8	0.40	−14	0	+	2
9	Cervical	3 mo.	5.8	2.0	0.22	−22	+	Positive histologically	4*
		5 mo.	6.0	1.9	0.08				
10	Thoracic	7 mo.	6.0	16.8	0.38	−11	0	0	3
11	Thoracic	5 mo.	5.1	7.1	1.39		0		3
12	Thoracic		5.7	7.7	0.48		0	0	2
13	Cervical	12 mo.	6.0	5.0	0.40	−20	+	+	3
14	Lumbar	3 yr.	6.4	13.5	0.39	Not obtained	Questionable history of mammary enlargement	+	2
15	Cauda equina	10 mo.	6.5	19.5	0.45	Not obtained	0	0	Cord not involved
16	Cervical	5 yr.	6.0	5.0	0.20	Not obtained	0	+	4

* Patient died during course of our study.

The urine of 7 patients was tested for estrogenic substance. In 6 of these cases the urine failed to reveal the presence of any estrogens. In the other case (Case 6), 16 rat units of estrogen were excreted in the urine in twenty-four hours (normal value 0 to 25 rat units in twenty-four hours).

GYNECOMASTIA AND TESTICULAR ATROPHY AFTER INJURY TO THE SPINAL CORD

Four of the 16 men had bilateral enlargement of the mammary glands. This incidence closely approximates that in a small series previously reported (3). The interval between trauma to the spinal cord and occurrence

of enlargement of the mammary gland was three, five, eleven and twelve months in Cases 9, 6, 7 and 13 respectively. In 3 cases gross testicular atrophy occurred as evidenced by testicular measurements of less than 4 to 5 cm. by 2.5 to 3 cm., and was markedly evidenced histologically in the fourth case at necropsy (Fig. 1). The occurrence of gynecomastia and other metabolic variations in our cases is tabulated in Table 2.

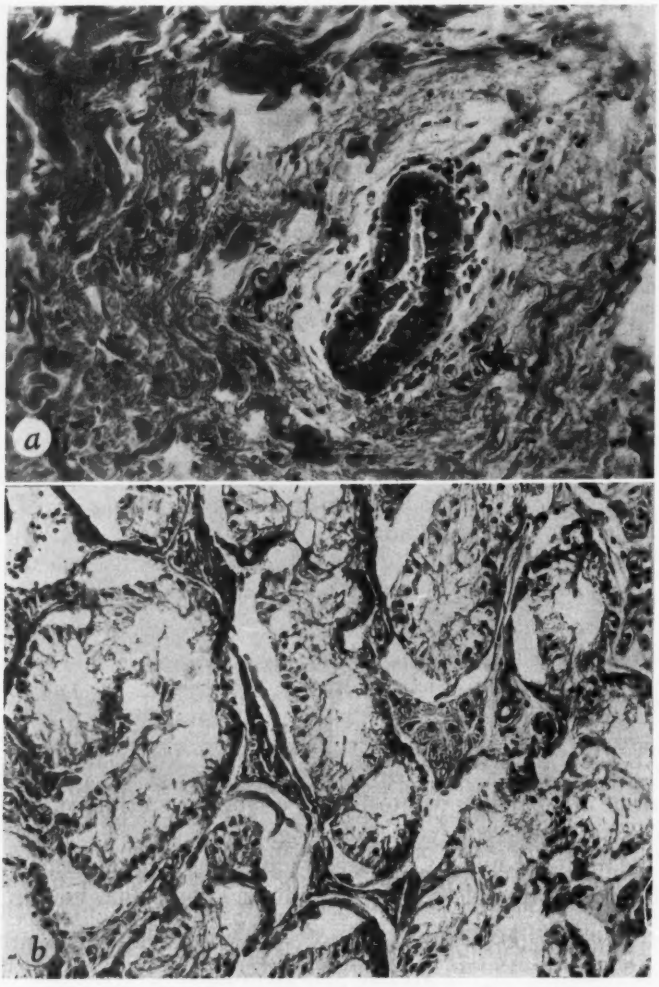

FIG. 1 *a*. Hyperplastic duct in enlarged breast (Case 9). The duct demonstrates papillomatous infoldings and is surrounded by loose edematous connective tissue (hematoxylin and eosin, ×200). *b*. Section of testis (Case 9) showing evidence of atrophy of the testicular tubules and aspermatogenesis (hematoxylin and eosin, ×140).

In 4 cases (Cases 1, 8, 14 and 16) in which gynecomastia did not develop, testicular atrophy could be demonstrated.

COMMENT

It is apparent from these observations that severe trauma to the spinal cord is followed by certain metabolic disturbances which seem to follow the catabolism of protein within the body and a markedly negative balance of nitrogen. During the catabolic period results of the bromsulfalein test may show impairment of liver function, but this impairment resolves spontaneously as the catabolic reaction subsides. The postacute and chronic periods may be notable in some instances for the development of gynecomastia, testicular atrophy, decreased urinary excretion of 17-ketosteroids and corticosteroids, decreased metabolic rate and hypoproteinemia. These metabolic phenomena may occur collectively or singly during the months succeeding severe trauma to the spinal cord of a male patient.

Any consideration of the pathogenesis of these phenomena must be frankly labeled as speculation. Selye (16) has considered the widespread effects of trauma on metabolic processes in his studies of the alarm reaction and adaptation syndrome, and he offered the opinion that the endocrine glands are primarily responsible for the catabolic reaction and other responses to "sudden stimuli to which the organism is not quantitatively or qualitatively adapted." Transection of the spinal cord has been demonstrated to provide an adequate stimulus for production of the alarm reaction (24). Because of early hypertrophy of the adrenal cortex during the alarm reaction, Selye has suggested that this gland plays a major role in the development of subsequent phenomena (16, 25). Although considerable evidence is at hand which suggests that the adrenal cortex is intimately concerned with metabolism of protein (26, 27, 28), an etiologic relation of the adrenal cortex to the post-traumatic catabolic reaction has not been established. Moreover, in a report concerning catabolism of protein after trauma in the rat, Noble and Toby stated that the "protein catabolism after trauma was apparently not dependent on the adrenal cortex" (29), a conclusion which also has been reached by other workers (30, 31). These latter workers have demonstrated that, although the secretion from the adrenal glands appears necessary in order for the catabolic reaction to occur, it is not essential that the secretion from these glands be increased. Wells and Kendall (32) and others (33) have demonstrated that the adrenal cortex and thyroid gland are synergistic in control of protein metabolism. The ultimate role assigned to these glands in the mechanism of the catabolic reaction must remain unanswered at this time.

With the evidence at hand, we cannot affirm or deny that changes in

the endocrine glands, secondary to their role in the alarm reaction, or changes in the function of the anterior pituitary gland at the expense of its gonadotropic elements, account for the later metabolic sequelae. Although atrophy of the anterior pituitary gland, prostate, testes and adrenal cortices has been reported after spinal cordectomy (4), the significance of these changes is not yet clear. Likewise, we cannot state whether or not denervation of any of the endocrine glands, secondary to the lesion of the spinal cord, is causally related to these phenomena. Bustamente (34) concluded from an investigation of the relationship of the hypothalamus to genital development, that there are direct nervous connections via the spinal cord between the hypothalamus and the genital organs. Troland and Brown (35) stated that genital change is not evident clinically after transection of the spinal cord. However, 8 of the 16 men in our series showed evidence of testicular atrophy. Consequently, the possibility of interruption of nervous pathways from the hypothalamus to the testicles by transection of the spinal cord must be considered.

Further speculation regarding the pathogenesis of these phenomena is beyond the scope of our report. The pathogenesis of gynecomastia in paraplegics has been considered briefly in previous communications (3, 4). The relation between trauma to the spinal cord and the development of metabolic disturbances, consisting in the acute period of protein catabolism and impaired liver function and followed by gynecomastia, testicular atrophy, decreased basal metabolic rate, lowered excretion of 17-ketosteroids and corticosteroids and hypoproteinemia invites further study before a sound hypothesis can be offered.

SUMMARY

The cases of 16 adult men who had experienced trauma to the spinal cord were studied. During the acute post-traumatic period those men who had undergone severe injury to the spinal cord demonstrated urinary excretion of large amounts of nitrogen (up to 29 Gm. daily), creatinuria and impaired liver function, as evidenced by the results of the bromsulfalein test for liver function. During the postacute and chronic periods, the course of certain of these patients was marked by lowered excretion of 17-ketosteroids and corticosteroids, gynecomastia, testicular atrophy, decreased metabolic rate and hypoproteinemia. Each patient had one or more of these symptoms and signs, the complete syndrome being noted in the cases of 4 of the 16 paraplegic men included in our study.

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ALTERATIONS IN TESTICULAR STRUCTURE AND FUNCTION IN ORGANIC DISEASE OF THE PITUITARY

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A STUDY was made of the gross and microscopic morphology of the testes in 20 patients with organic pituitary disease of from one to twenty-four years' duration. The group ranged in age from 16 to 67 years, and included cases of cystic and solid craniopharyngiomas, eosinophil and chromophobe adenomas, cases of destruction of the pituitary by infiltrating extra-pituitary tumors and 1 case of pituitary atrophy following surgical trauma.

METHODS

Urinary gonadotropins (follicle-stimulating hormone, FSH) were measured by the method of Klinefelter, Albright and Griswold (1). The normal range in adult males is between 13 and 105 mouse units per twenty-four hours.

Excretion of 17-ketosteroids was measured by the technic of Bowman (2). The normal range for men is 5 to 15 mg. per twenty-four hours.

The water excretion test of Robinson, Power and Kepler (3) provides an index of adrenal cortical function. Normal values range upward from an index of 25.

The ACTH test measures the ability of the adrenal cortex to respond to 25 mg. of adrenocorticotropic hormone given intramuscularly (4). The criteria of a normal response are a decrease in circulating eosinophils greater than 50 per cent and an increase of 50 per cent or more in urinary uric acid excretion rate.

RESULTS

The findings on study of the testis biopsy material are correlated in Table 1 with the nature and duration of the pituitary lesion and with the urinary excretion of gonadotropins and 17-ketosteroids. The protocols in the concluding section record clinical details which do not lend themselves to tabular presentation.

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TABLE 1

No.	Age (yrs.)	Pituitary lesion and estimated duration (yrs.)		FSH m.u./24 hrs.	17-KS mg./24 hrs.	Genital size		Seminiferous tubules					Basement membrane thickness	Leydig cells	
						Prost.	Test.	Size	Gametogenic cells		Sertoli cells			Number	Character
									Number	Maturity	Number	Character			
1	25	Craniopharyngioma	5	105–212	11.2	N	N	N	++++	++++	++++	N	N	++	S
2	43	Chromophobe adenoma	1	26–52	—	N	N	N	++++	++++	++++	N	N	++	N
3	39	Chromophobe adenoma	1	13–105	—	N	N	N	++++	++++	++++	N	N	++++	N
4	47	Chromophobe adenoma	2	13–105	—	S	N	N	+++	+++	++++	vacuolated	sl. thick	+++	oxyphilic
5	33	Eosinophil adenoma	6	13–26	6.3	L	L	N	++++	++++	++++	N	N	++++	N
6	42	Eosinophil adenoma	15	N (a)	N(b)	N	N	N	++++	++++	++++	N	N	+++	vacuolated
7	43	Eosinophil adenoma	13	52–105	11.9	N	N	N	++++	++++	++++	N	N	+++	N
8	28	Eosinophil adenoma	5	6–26	7.2	S	N	S	++	++	++++	N	m. thick	0	—
9	16	Malignant adenoma	1	–6	3.3	S	S	S	+	+	+++	vacuolated	sl. thick	++	atrophic
10	16	Craniopharyngioma	4	–6	0.9	S	S	S	+	+	+++	N	m. thick	+	atrophic
11	59	? Atrophy	3	6–13	1.2	L	S	S	++	+++	++++	vacuolated	sl. thick	+	granular
12	44	Eosinophil adenoma	5	–6	12.9	S	S	S	++	+++	++++	N	sl. thick	+++	granular
13	17	Oligodendroglioma	7	—	—	N	S	S	+	++	++++	vacuolated	N	+	atrophic
14	33	Craniopharyngioma	5	13–26(c)	2.6	S	S	S	+	+	++++	S	sl. thick	0	—
15	67	Craniopharyngioma	24	–6	0.8	v. S	v. S	S	0	—	0	(complete	fibrosis)	0	—
16	43	Chromophobe adenoma	16	–13	1.8	S	S	S	+	+	+++	vacuolated	v. thick	0	—
17	26	Infiltrating pinealoma	2	–6	1.1	v. S	v. S	S	+	+	++	S	m. thick	0	—
18	22	Pituitary dwarf with hypogonadism	10?	–13	2.6	S	S	S	0	—	+++	vacuolated	v. thick	0	—
19	24	Suprasellar oligodendroglioma	10?	N(a)	—	S	S	S	+++	++	+++	N	sl. thick	+	poorly developed
20	53	Atrophy following surgical trauma	3	–6	3.2	S	S	v. S	0	—	++	vacuolated	v. thick	+	poorly developed

KEY: S—small; N—normal; L—large
sl.—slightly; m.—moderately; v.—very
(a) By Friedman test
(b) Androgen assay (Koch)
(c) 18 mos. before testis biopsy

With respect to the number or maturity of cells:
++++ Normal average
+++ Slightly reduced
++ Moderately reduced
+ Markedly reduced
0 Absent

DISCUSSION

From a study of the data in Table 1 it is possible, on the basis of the histologic structure of the seminiferous tubules, to divide the cases into two groups: those with essentially normal tubular structure (Cases 1 to 7 inclusive) and those with definite histologic evidence of tubular abnormality (Cases 8 to 20 inclusive).

It may be noted that, ordinarily, normal-sized testes were found on biopsy to contain normal-sized tubules. Normal-sized testes in some cases, however, were found to contain subnormal numbers of Leydig cells. With respect to the character of Leydig cells, the presence of granularity was interpreted as representing degenerative change rather than "presecretory granulation." This is in no way specific, as granulation of the interstitial cells is common in elderly men (5). The terms "oxyphilic" and "vacuolated" refer to changes interpreted by us as degenerative in nature.

The clinician's estimate of the size of the prostate, although subject to considerable error, correlates fairly well with the number and character of the Leydig cells. The principal exception to this observation (the patient in Case 2) had symptoms suggestive of benign prostatic hypertrophy for several years before the onset of pituitary insufficiency. Thus the large prostate could be regarded as the residuum of an even larger gland undergoing shrinkage as increasing pituitary insufficiency led to impaired Leydig cell activity.

The size and number of the seminiferous tubules showed good correlation with the urinary excretion of FSH. One apparent exception is Case 14, but in this instance FSH assay was not done at the time testis biopsy was performed, and the relatively normal level quoted was determined eighteen months previously and probably should be disregarded when interpreting the other data.

Urinary excretion of 17-ketosteroids has proved an unreliable index of Leydig cell activity, an observation in accord with that of numerous investigators. In the group under consideration, low excretion of 17-ketosteroids (less than 4.0 mg. per twenty-four hours) is seen only in the patients who evidence adrenocortical insufficiency in addition to Leydig cell failure. This is in agreement with a previous observation that urinary 17-ketosteroid excretion averages about 50 per cent of normal when adrenocortical failure or Leydig cell failure occurs separately (6).

Finally, in the group as a whole, careful histologic study failed to reveal any morphologic or tinctorial characteristics of the biopsy material which would permit differentiation of primary testicular failure from that secondary to pituitary insufficiency.

Relation of the type of pituitary lesion, the age and general condition of the patient, to the testicular changes found:

Case 1 concerns a young man who had hypopituitarism as indicated by short stature, and poor sexual development as well as by laboratory tests. On first examination, urinary FSH levels were too low to read. Later, when pressure was removed from his pituitary, gonadotropin levels rose to above normal. Subsequently, when moderate spermatogenic activity was evident and the testes approached normal histologically, the gonadotropin levels also fell to normal.

In Case 2 there were only local signs to indicate this patient's pituitary lesion, and the fact that there were no symptoms of failure appears to correlate well with his normal gonadotropin assay and normal testicular structure.

In Case 3 also, only local signs indicated the condition, although the patient had a normal gonadotropin assay, normal testicular structure, and a sperm count and semen volume which approached normal.

Case 4 presented more definite clinical evidence of pituitary deficiency. In this case, however, some thickening of the basement membrane was present in the testes. Whether the discoloration of the Leydig cells is part of a normal aging process is difficult to ascertain. It is noteworthy that the 2 other patients (Cases 11 and 12) who had granular pigmentation of Leydig cells were aged 59 and 44 respectively, whereas some younger patients with more severe atrophic changes (Cases 10, 13 and 19) showed no granulation.

Cases 5 to 8 inclusive and Case 12 were instances of acromegaly. These men cannot be considered as having pituitary failure comparable to that of most other patients presented. In Case 5, for example, the sperm count was 432,000,000, the semen volume approximately normal, and the testes normal. In Case 6 the patient must be similarly judged as having normal gonadotropin production despite the eosinophil tumor. It is interesting that under these circumstances sexual power had diminished.

In Case 7 the situation was almost identical, the semen volume and sperm count being normal. In this patient, as in the other acromegalics observed, the urinary FSH could be reduced below normal by stilbestrol therapy. In Case 8 the concurrent diabetes may possibly have been a factor in producing some testicular change.

The patient in Case 11 was not shown to have a pituitary tumor and although his tests indicate pituitary failure almost beyond doubt, both the gonadotropin assays and testicular changes are less severe than in most of the other cases (9 to 20). In Case 12, the diabetes may have been a factor in aggravating degenerative changes in the testes. The low gonadotropin assay was present before the diabetes was brought under satisfactory control. It is debatable whether the relative malnutrition produced by the diabetes

in this patient may have resulted in a reduction in urinary FSH which was short-lived enough to permit the preservation of fairly good testicular structure.

In Case 18 the patient was not shown to have pituitary tumor and was judged to be deficient in anterior lobe function, chiefly on the basis of his small stature, low excretion of FSH and 17-ketosteroids and his low basal metabolic rate.

In Cases 9, 10, 13, 15, 16, 17, 18 and 20 the patients all were judged clinically as having relatively severe pituitary failure and observations appear to confirm this opinion.

In general, those patients with clinical evidence of severe pituitary failure show the lowest urinary gonadotropin levels and most severe alterations in testicular structure. As testicular damage progresses spermiogenesis appears to be effected first, then further damage is indicated by a decrease in the number of spermatids. Finally, there is a virtual disappearance of spermatocytes. Slight to severe thickening of the basement membranes of the tubules accompanies these changes. The Leydig cells gradually become reduced in size and in number and eventually disappear. Brownish granular pigmentation has been noted in this series, only in the Leydig cells of those patients over 44 years of age. Except in instances of the most extreme testicular fibrosis the Sertoli cells are relatively spared.

SUMMARY

Observations are presented on the histology of the testes in 20 cases of organic pituitary disease and these findings are correlated with the size of the gonads and prostate, urinary gonadotropin and 17-ketosteroid excretion, and in some cases, with semen examination.

The following general observations were made:

1. Testicular size appears to be determined by the size and number of the seminiferous tubules.

2. The degree of tubular damage is related to the degree of deficiency of pituitary gonadotropin stimulation as reflected by urinary gonadotropin (FSH) excretion.

3. The size of the prostate gland correlates fairly well with the number and quality of Leydig cells present.

4. Changes in the numbers and physical integrity of Leydig cells cannot be accurately inferred from urinary excretion of gonadotropin (FSH) or 17-ketosteroids.

5. In the testis biopsy material there were no histologic characteristics noted which would permit differentiation of primary testicular failure from that secondary to pituitary failure.

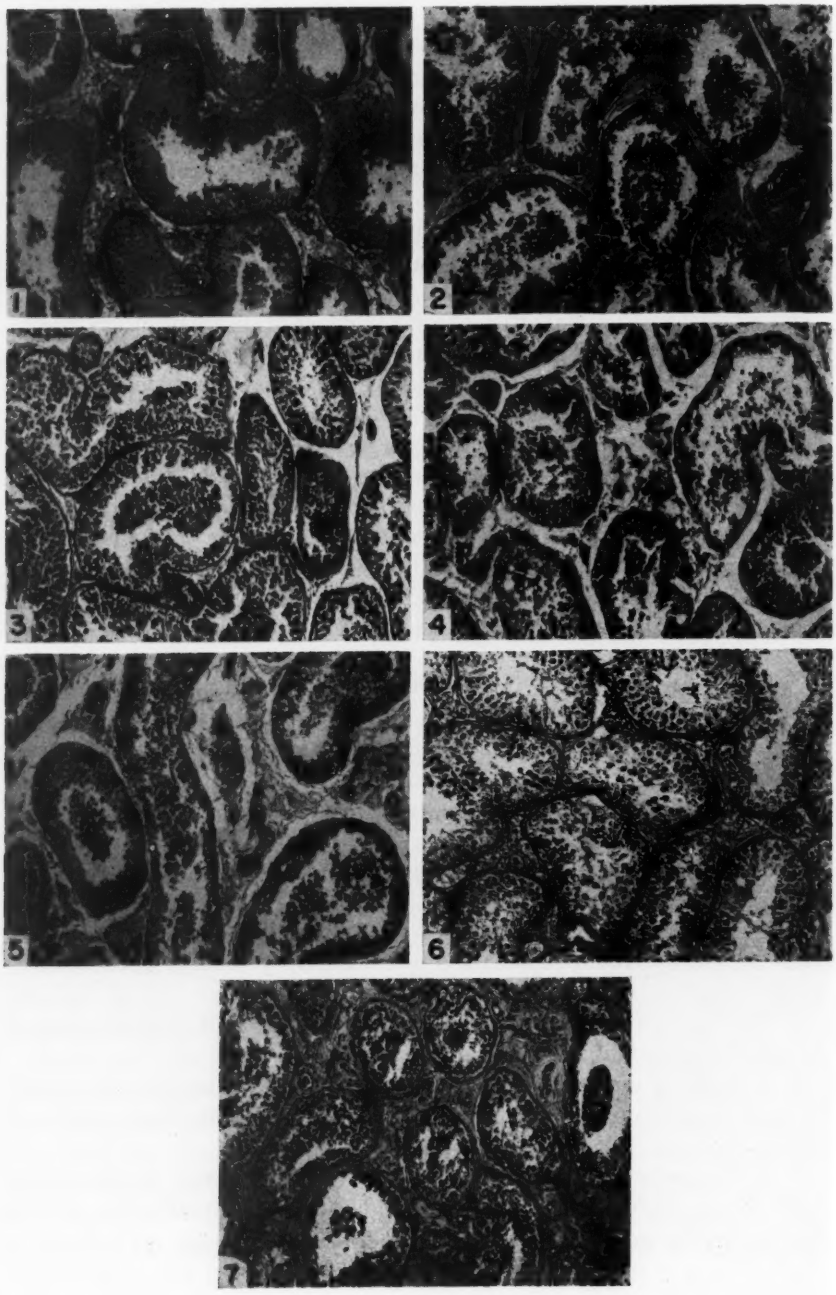

PLATE I

6. There appeared to be no histologic changes in the testes characteristically associated with the presence of one type of pituitary lesion as opposed to another.

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PROTOCOLS

Case 1. This man presented himself at age 18 with a five-year history of increasing bitemporal headaches, occasional diplopia, blurring of vision and failure to gain in weight and height. The genitalia had shown no appreciable development since the age of 13 and erections and nocturnal emissions had ceased about three years previously. Examination showed a height of 60 inches, span 66 inches, bitemporal hemianopsia with constricted nasal fields, no beard, scanty body hair with female type distribution, a high pitched voice, small testes and prostate, but a bone age of 17 to 18 years. Urinary gonadotropin excretion was too low to measure at that time, the basal metabolic rate was minus 26 per cent and the insulin tolerance test showed hypoglycemia unresponsiveness. A craniotomy was performed, a large suprasellar cyst was drained and part of the cyst wall and an adjacent adenoma were removed. Postoperatively there was gradual improvement in general health, secondary sex characteristics resumed development and genitalia attained adult proportions. There was only slight gain in stature, however, in seven years of observation after operation, and beard growth was not sufficient to warrant shaving. At this time urinary FSH excretion was between 105 and 212 mouse units per twenty-four hours, urinary 17-ketosteroid excretion was 11.2 mg. per twenty-four hours and semen examination indicated 27,000,000 nonmotile spermatozoa in a 1.7 cc.

PLATE I. Cases 1–7. Histologic sections of the biopsy specimens show essentially normal-appearing tubules and tubular cells. In Cases 1, 3 and 5 the total spermatozoa counts were 27, 59, and 932 millions, respectively. These noticeable variations in spermatogenic capacity would not readily be inferred from the histologic appearances.

In only 1 of the group was there evidence of Leydig cell insufficiency, as indicated by small prostatic size. In this instance (Case 4) the Leydig cells were numerous but they showed a severe oxyphilia which presumably reflects the changes which led to functional insufficiency of these cells. Conversely, in Cases 1 and 2, although Leydig cells were few in number, they were functionally adequate to maintain prostatic size.

specimen. Testis biopsy material revealed tubules of average size with evidence of fairly active spermatogenesis and a basement membrane of average thickness. The interstitial tissue was of average density and contained a few poorly developed Leydig cells. The most recent assay of urinary gonadotropins (about nine years after operation) showed from 13 to 105 mouse units per twenty-four hours.

Case 2. This patient was first observed at the age of 43 with a history of headaches, blurring of vision, diplopia and narrowing of the visual fields which had developed over the period of a year. Examination revealed severe field defects and there was roentgenographic evidence of extensive erosion of the sella. Following craniotomy, subtotal removal of a chromophobe adenoma was carried out. Just prior to operation the urinary gonadotropin excretion was between 26 and 52 mouse units per twenty-four hours. The testes and prostate were grossly normal. Histologically the testis biopsy material showed normal tubular structure but pronounced diminution in the number of Leydig cells.

Case 3. The patient was first seen at age 39 with a one-year history of visual disturbances and loss of libido. The basal metabolic rate six months previously was reported to have been minus 21 per cent. A diagnosis of pituitary neoplasm was made on the basis of visual field defects and roentgenographic evidence of erosion of the sella. Following removal of a chromophobe adenoma there was improvement in vision, slow gain in weight and strength but no improvement in libido or potentia. Clinically there was judged to be a mild degree of pituitary failure. The urinary gonadotropin excretion was 13 to 105 mouse units per twenty-four hours; the basal metabolic rate, minus 25 per cent; water excretion index, 23; the ACTH test gave a normal response, and semen examination indicated 59,000,000 spermatozoa in a 1.7 cc. specimen. The testis biopsy specimen showed essentially normal structure.

Case 4. This 47-year-old man had a two-year history of progressive headaches, weakness, loss of body hair, decreased libido, and failing vision. Examination showed a blood pressure of 106/72, atrophic skin, sparse beard, soft testes of normal size but a small prostate gland. Erosion of the sella turcica was evident on x-ray study. Gonadotropin excretion was within normal range (between 13 and 105 mouse units) on several occasions. The diagnosis of chromophobe adenoma was confirmed at operation. Testis biopsy material showed tubules of average size with slight reduction in spermatogenesis and a relative increase in size and vacuolation of Sertoli cells. There was slight thickening of the basement membrane. Small clumps of oxyphilic Leydig cells were present in the interstitial tissue.

Case 5. This patient was seen at age 33 with a six-year history of gradually developing fatigability, weight loss, polydipsia, polyuria, fainting attacks and tingling in hands and feet. The sella was enlarged and the clinical features of acromegaly were present. The urinary gonadotropin excretion was between 13 and 26 mouse units per twenty-four hours, urinary 17-ketosteroids 6.3 mg. per twenty-four hours, basal metabolic rate plus 52 per cent and radioactive iodine tracer uptake 10 per cent. The testes and prostate were moderately large and the semen contained 432,000,000 spermatozoa in a 2.4 cc. specimen. Testicular biopsy material showed essentially normal structure histologically.

Case 6. This patient, 42 years of age, had typical acromegaly. Changes in the skeleton had been present nine years and libido and potentia had been diminished for two years. In addition, there was a large substernal goiter causing tracheal compression and venous engorgement over the neck and chest. X-ray therapy to the pituitary area relieved the headaches temporarily but skeletal changes advanced and the blood pressure tended to increase. The patient died at age 47 following an operation to relieve tracheal compression by the thyroid mass. Urinary gonadotropin excretion as measured by the Friedman

test was normal six months before operation but was not measurable just prior to operation. Androgen assays six months before surgery gave normal values. The basal metabolic rate was plus 9 per cent. The testes and prostate were normal in size on clinical examination. At autopsy an eosinophilic adenoma, 1.3 cm. in diameter, was found in the left half of the anterior pituitary. The prostate weighed 40 Gm. and the testes 15 and 20 Gm. respectively. Histologically the testes were normal except for a slight decrease in interstitial tissue and some vacuolation of Leydig cells.

Case 7. This 43-year-old man was seen with a 13-year history of painful hands and feet. On questioning, he admitted noting enlargement of the head, nose, and mandible, some generalized weakness, and diminished libido and potentia. Examination indicated typical acromegalic skeletal and soft tissue changes and enlargement of the sella, but no constriction of visual fields. The testes, penis, and prostate were normal in size. FSH excretion was between 52 and 105 mouse units per twenty-four hours initially but after one month of therapy with 3 mg. of stilbestrol daily it had decreased to less than 6 mouse units. The 17-ketosteroid excretion was 11.9 mg. per twenty-four hours. The total sperm count was 300,000,000 in 3.8 cc. of ejaculate initially, but after two months of stilbestrol therapy the ejaculate had declined to a few drops of thick mucoid material. Testis biopsy material taken before stilbestrol therapy showed essentially normal structure with active spermatogenesis but a slight decrease in the number of Leydig cells.

Case 8. Symptoms of headache, loss of strength, decreased visual acuity in the right eye, loss of libido and increasing size of hands and feet began to appear in this patient at the age of 23. Three years later, when the patient was first observed, a diagnosis of acromegaly readily was made on clinical grounds and an eosinophilic adenoma of the pituitary was removed. Improvement in vision and cessation of growth of the hands and feet followed operation but the headaches persisted. Two years after operation the patient suddenly developed diabetes which required 70 units of insulin per day. At that time FSH excretion ranged from less than 6 to about 26 mouse units per twenty-four hours, urinary 17-ketosteroid excretion was 7.2 mg. per twenty-four hours and the basal metabolic rate was minus 16 per cent. The testes were normal in size but the prostate was judged to be smaller than normal. Testicular biopsy material showed small tubules, some of which contained a fair number of spermatogonia, and others a predominance of Sertoli cells. Mitoses were numerous and there were a few spermatocytes but no spermatozoa evident. The basement membrane was moderately thickened. No Leydig cells were found.

Case 9. The history of this patient's illness began at age 16 with weight loss, polyuria and vomiting. Examination revealed hypogonadism, emaciation (height 65 inches, weight 87 pounds) pallor, hypotension, dry skin, edema of the optic discs, and a spinal fluid pressure of 215 mm. X-ray and visual field studies suggested a suprasellar lesion, which was confirmed at operation. Histologically the tumor was an atypical chromophobe adenoma with cytologic evidence suggesting malignant change. Functional insufficiency of the anterior pituitary was indicated by a urinary FSH excretion of less than 6 mouse units per twenty-four hours, a 17-ketosteroid excretion of 3.3 mg. per twenty-four hours, a basal metabolic rate of minus 38 per cent and a water excretion index of 10. Grossly the prostate was small and the testes small and soft. Testicular biopsy specimens obtained prior to the craniotomy showed tubules of reduced size, formed principally of Sertoli cells and with a few spermatogonia, some of which showed mitosis. Interstitial tissue was decreased in amount but of increased density and contained a few atrophic Leydig cells.

Case 10. A diagnosis of pituitary tumor was made at the age of 14 on the basis of right temporal headaches for two years, retardation of growth rate for four years and a

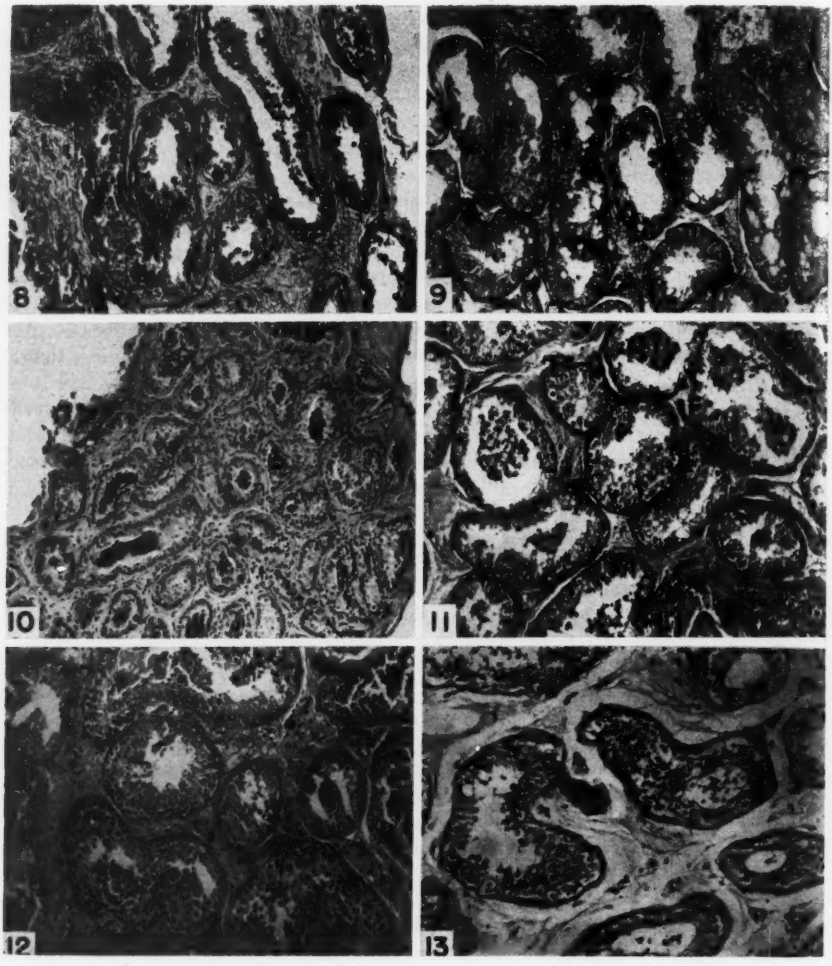


PLATE II. Cases 8–13. For description, *see* Plate III.

temporal field defect in the right eye of short duration. The patient's height was 5 feet, 3 inches at age 10; and 5 feet, 7 inches at age 14. His weight was 100 pounds at age 10, 130 pounds at age 12, and 100 pounds at age 14. The patient received x-ray therapy to the pituitary area but symptoms persisted and at the age of 16 there was weight loss, weakness, underdeveloped genitalia, absence of secondary sex characteristics, severe headache, vomiting and early optic atrophy on the right side. At this time urinary FSH excretion was less than 6 mouse units per twenty-four hours and 17-ketosteroid excretion 0.9 mg. per twenty-four hours. Surgical evacuation of a cystic craniopharyngioma was done and a testis biopsy performed. Histologically the tubules were small and composed almost entirely of Sertoli cells with a few spermatogonia resembling those seen

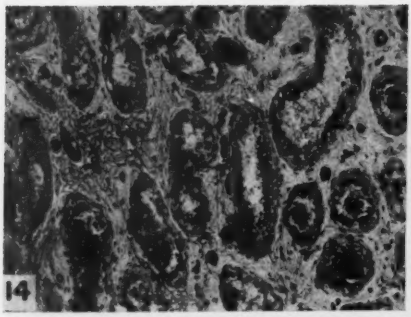
in a boy of about 11 years of age. The basement membrane was considerably thickened. The interstitial tissue was dense and contained only a few atrophic Leydig cells.

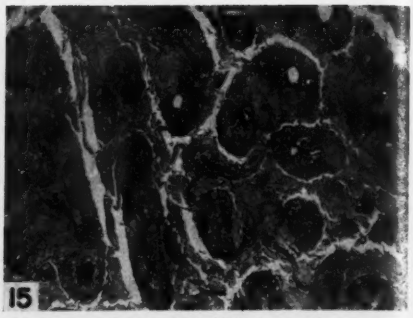
Case 11. A 59-year-old man presented himself with the following complaints: progressive weakness and fatigability over a period of three years, loss of libido and potentia and diminution in size of external genitalia for two years, and hot flashes and intolerance to cold for the same length of time. Blood pressure was 118/74, the skin was atrophic and of "pasty" color, body hair sparse, testes and penis small but the prostate somewhat hypertrophied. The sella was not enlarged and the visual fields showed only slight symmetrical constriction. Other studies indicated a urinary gonadotropin excretion between 6 and 13 mouse units per twenty-four hours, a 17-ketosteroid excretion of 1.2 mg. per twenty-four hours, a basal metabolic rate of minus 33 per cent, and a water excretion index of 1.1. The ACTH test was suggestive of adrenal insufficiency (eosinophils increased 3 per cent, uric acid/creatinine ratio increased plus 43 per cent). Histologically, the testes showed tubules of slightly reduced size with a moderate reduction in gametogenic elements and prominence of vacuolated Sertoli cells. The basement membrane was slightly thickened and the interstitial tissue contained a few small Leydig cells with increased brownish granularity of the cytoplasm.

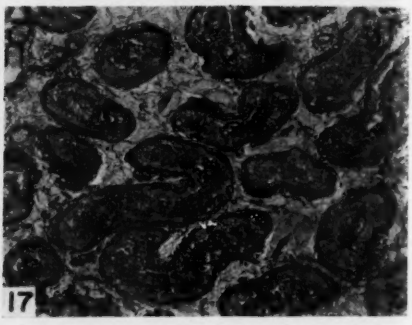
Case 12. This patient was seen first at the age of 44 with signs and symptoms of acromegaly which had developed over a period of five years. Libido had been absent for one year and polyphagia, polydipsia, and polyuria present for six months. The finding of glycosuria and hyperglycemia corroborated the suspicion of diabetes. Visual field defects and enlargement of the sella enjoined the use of radiation to the pituitary area and stilbestrol therapy. Prior to therapy, urinary gonadotropin excretion was too low to be measured and 17-ketosteroid excretion was 12.9 mg. per twenty-four hours. A semen examination was not possible because of impotence. The testes were small in size and histologically showed small-sized tubules lined by sustentacular cells and a reduced number of germinal cells showing fairly active spermatogenesis. The basement membrane was slightly thickened. There was some increase in interstitial tissue and moderate numbers of granular Leydig cells were present.

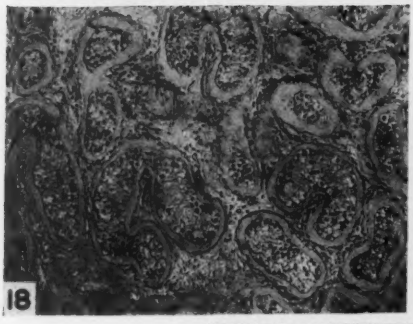
Case 13. This patient was first observed at age 17 with a seven-year history of attacks of temporal and occipital headache and drowsiness, which dated from head injuries received in an automobile accident. He had been a "fat" boy until the onset of adolescence, which had been delayed and was proceeding slowly. His voice was high pitched. He had erections but no libido and body hair was almost absent. The sella was enlarged and the posterior clinoids eroded. There was slight temporal contraction of the visual fields and both discs showed blurred margins. The basal metabolic rate was minus 23 per cent. Linear calcified streaks in the suprasellar area suggested that the evidence of pituitary failure probably resulted from an extrasellar mass. Examination indicated ataxia, optic atrophy, increased spinal fluid pressure and bilateral hydrocephalus. Craniotomy at this time showed no definite tumor but reddish-grey tissue enclosed a cystic space beneath the floor of the third ventricle. The patient died as the result of postoperative edema of the brain. Autopsy showed flattening of the pituitary due to the pressure exerted by an oligodendroglioma. The prostate weighed 30 Gm. and the testes 14 Gm. each. Histologically the spermatogenic cells were noticeably reduced in number but in a few areas there was maturation to the spermatid stage. Sertoli cells, which showed vacuolization, appeared relatively increased in number. The basement membranes were normal or slightly thickened. The interstitial tissue was loosely arranged, relatively increased in amount, and contained few Leydig cells.

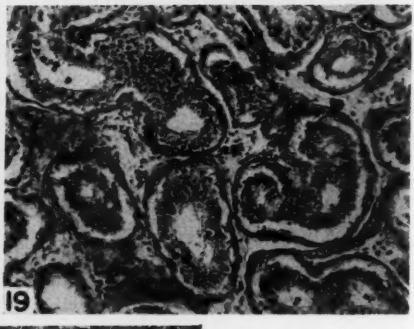
Case 14. This patient was observed at age 29 with a nine-month history of anorexia, fatigability, and a weight loss of 45 pounds. Mild hypertension and a small prostate











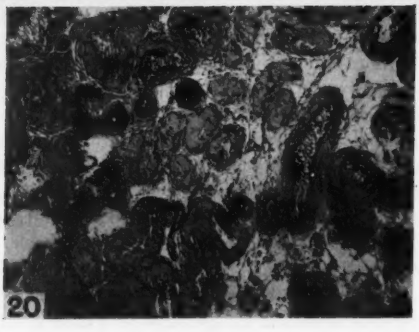


PLATE III

were the only findings at this time. Eighteen months later the patient was seen again because of bitemporal headaches and roentgenograms revealed early erosion of the left posterior clinoid. An internal carotid aneurysm was suspected but excluded by craniotomy. The patient was again observed about four years after the first visit and a diagnosis of pituitary adenoma made on the basis of failing libido and potentia, loss of body hair, temporal field defects, and further erosion of the clinoids. Treatment with desoxycorticosterone acetate (DCA), testosterone propionate, and radiation to the pituitary was carried out for four years, after which further constriction of the visual fields forced an attempt at surgical removal of the mass. A cystic craniopharyngioma was evacuated but the patient died a few days postoperatively. Two years before death the urinary gonadotropin excretion was about 26 mouse units and 17-ketosteroid excretion was 2.5 mg. per twenty-four hours. One year before testis biopsy was performed the water excretion index was 4.5 and the basal metabolic rate minus 12 per cent. Grossly the testes and prostate were about one-half normal size. Histologically the testes showed small tubules with severe decrease in spermatogenic cells and slightly thickened basement membrane. Some tubules were lined by a single layer of Sertoli cells. The interstitial tissue was increased in amount and density and there were no Leydig cells apparent.

Case 15. This 67-year-old man had a suprasellar cyst removed at age 43 by Dr. Harvey Cushing. Postoperatively there remained partial blindness and mild diabetes insipidus, but symptoms of hypopituitarism were mild enough to be well-tolerated until the age of 67, when help was sought for increasing fatigability and "lack of drive." Clinically there was myxedema, testicular and prostatic atrophy, aspermia, and a remarkably pale sallow complexion. Anterior pituitary insufficiency was corroborated by the finding of a urinary gonadotropin excretion of less than 6 mouse units per twenty-four hours. The basal metabolic rate was minus 18 per cent, urinary 17-ketosteroid excretion 0.8 mg. per twenty-four hours, water excretion index 2.2, and the response to the ACTH test was subnormal. Testicular biopsy specimens showed extensive fibrosis of both tubules and interstitial tissue with complete loss of testicular architecture.

Case 16. This patient was first seen at the age of 43 with a 16-year history of intermittent painless icterus, gradually increasing weakness and fatigability, decreased libido and potentia, and aspermia. On examination the skin was sallow and of fine texture, and there was diminution of body hair. Testes were small and of soft consistency and the prostate barely palpable. The sella was enlarged but there was no visual field defect. Urinary gonadotropin excretion was between 6 and 13 mouse units per twenty-four hours and the 17-ketosteroid excretion was 1.8 mg. per twenty-four hours. The basal

PLATE III. Cases 14–20. The histologic sections of the biopsy specimens in Cases 8 through 20 show moderate to severe degrees of tubular and interstitial cell degeneration. The extreme limits of variation in this group are represented by Cases 12 and 15, respectively. It is of interest to note that a rough correlation exists between the degree of thickening of the tubular basement membrane and the degree of germinal cell degeneration. Whether the basement membrane thickening precedes and contributes to the epithelial degeneration by impeding transfer of nutriments, or whether it follows and results from epithelial degeneration, or merely accompanies the epithelial changes, is not yet established. Nearly half the cases of this group showed no Leydig cells and the remainder showed Leydig cells which were both quantitatively and qualitatively deficient.

In all but Case 11 there was insufficient androgen production to maintain prostatic size. The superior durability of the Sertoli cells, which is one of their generally recognized characteristics, is evident in their tendency to persist in the tubules of this group.

metabolic rate was minus 21 per cent, glucose tolerance was normal, and the ACTH test showed a 44 per cent decrease in circulating eosinophils and an increase of 241 per cent in the urinary uric acid/creatinine ratio. Semen could not be obtained for examination. Testis biopsy material showed small tubules lined by vacuolated Sertoli cells and containing only rare spermatogonia. The interstitial tissue showed dense hyalinization and contained no Leydig cells.

Case 17. A 26-year-old Japanese patient was seen with a one-year history of polyphagia and diabetes insipidus and a one-month history of occipital headaches, dizziness, decreased appetite and endurance. Examination disclosed hypotension and small genitalia. Urinary gonadotropin excretion was less than 6 mouse units and 17-ketosteroids 1.1 mg. per twenty-four hours. The water excretion index was 6.4 and the basal metabolic rate ranged between minus 12 and minus 46 per cent. A diagnosis of pituitary insufficiency was made and replacement therapy attempted with pitressin, testosterone, thyroid, DCA, and sodium chloride, but the clinical condition deteriorated steadily and the patient died about two years after the onset of symptoms. Autopsy indicated a pinealoma extending into the third, fourth and lateral ventricles and involving the hypothalamus and pituitary. The pituitary showed a predominance of chromophobe cells with moderate reduction in oxyphils and total absence of basophilic cells. There was atrophy of thyroid, adrenals, testes, prostate and seminal vesicles. The testes weighed 8.5 Gm. each and were composed of small nonluminated tubules containing predominantly Sertoli cells. The basement membrane was thickened irregularly and showed a scalloped outline in cross section. There was a moderate amount of dense interstitial tissue but no Leydig cells were apparent.

Case 18. A patient, age 22, presented himself for observation with the chief complaint of small stature. His height was 5 feet, 2 inches, weight 89 pounds, and his habitus was of the asthenic type. Body hair was absent except for scanty hair growth on the pubic area, the voice was high pitched, the testes, penis, and prostate were small, erections were infrequent and no nocturnal emissions had occurred. The sella was normal in size and visual fields were intact. Bone age was 17 years. Urinary excretion of gonadotropins was less than 13 mouse units per twenty-four hours, 17-ketosteroid excretion was 2.6 mg. per twenty-four hours, and the basal metabolic rate was minus 35 per cent. The clinical diagnosis was anterior pituitary failure with dwarfism, hypogonadism, and hypometabolism. Testis biopsy material showed small tubules with severe fibrosis and hyalinization of the basement membrane. The tubules contained vacuolated Sertoli cells but no evidence of spermatogenesis beyond the stage of the occasional primary spermatocyte. Interstitial tissue was dense and showed fibrosis but no well developed Leydig cells were seen.

Case 19. A 24-year-old truck driver was seen first with a 10-year history of intermittent right frontal headaches, nausea and vomiting, and an episode of loss of consciousness. He was of asthenic habitus with fine skin and scanty body hair. The prostate and external genitalia were about one-third normal size. There was a left homonymous hemianopsia and bilateral edema of the optic discs. X-ray examination indicated depression of the floor of the sella, erosion of the clinoids, suprasellar calcification, and convolutional pressure atrophy of the skull. The basal metabolic rate was minus 18 per cent. Urinary excretion of gonadotropin, as indicated by the Friedman test, was low. Further laboratory studies were not available on this early case. The diagnosis of suprasellar tumor was made during craniotomy. A solid suprasellar mass the size of a hen's egg was found. The tumor, which extended posteriorly to the pons, was removed piecemeal and proved to be an oligodendroglioma. The patient died two days postoperatively and at

autopsy the pituitary showed evidence of mechanical compression and contained fewer eosinophils than usual. The adrenals showed some cortical hyperplasia. Histologically, the testes contained small tubules with slightly thickened basement membranes. The spermatogenic epithelium showed numerous mitoses but failure of maturation beyond the secondary spermatocyte stage. The interstitial tissue was of average density and contained a few poorly developed Leydig cells. The over-all appearance of the testis biopsy material suggested immaturity of the degree usually seen in a boy of about 11 years of age.

Case 20. This patient was first seen at the age of 58 by the ophthalmologist because of severe exophthalmos, chemosis, and conjunctivitis which had appeared one year previously following thyroidectomy for hyperthyroidism. In spite of various therapies with x-ray, iodine, thyroxine, and conjunctival drainage, the exophthalmos and edema progressed steadily and vision became impaired. An orbital decompression operation was performed and at the same time the anterior lobe of the pituitary was fulgurated in an attempt to reduce the supposed pituitary stimulus to exophthalmos. Following operation, exophthalmometer readings indicated regression of proptosis from a reading of 30 mm. to 25 mm. However, in the three months subsequent to operation the patient developed manifestations of adrenocortical insufficiency together with impotence and hypothyroidism. He was maintained on thyroid, testosterone, DCA and high salt intake but the signs and symptoms of pituitary failure became steadily more evident.

Three years after operation there was pronounced weakness, emaciation, loss of body hair, atrophy of testes and prostate, localized myxedema, and a blood pressure of 100/70. Urinary gonadotropin excretion was less than 6 mouse units per twenty-four hours, 17-ketosteroid excretion was 3.2 mg. per twenty-four hours, the basal metabolic rate was minus 21 per cent, the Kepler water excretion index 17 units, and the insulin tolerance test showed hypoglycemia unresponsiveness. Testis biopsy material showed small tubules with thick basement membrane. There was no germinal epithelium evident and the tubules were lined only by vacuolated Sertoli cells. The interstitial tissue contained a few poorly developed Leydig cells.

PHYSIOLOGIC EFFECTIVENESS OF ORAL PROGESTERONE*

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IT HAS been assumed for many years that ingested progesterone is physiologically ineffective because of partial destruction by gastric secretions and direct absorption into the portal circulation with subsequent immediate conjugation and deactivation in the liver. This belief has apparently been perpetuated on the basis of experimental studies in animals. The literature fails to disclose adequate studies of the physiologic action of progesterone administered orally to the human patient. Extensive search for some mode of administration other than parenteral has led to the synthesis of anhydrohydroxyprogesterone. Progesterone prepared in a vehicle suitable for absorption from mucous membranes has proved effective (1). Bickers (2), in a study of the comparison of intramuscular, oral and sublingual routes of administration of progesterone and anhydrohydroxyprogesterone, recently reported the results of six cycles during which oral free progesterone was administered in doses of 80 mg. per day for five days without estrogen priming. In none of the six cycles did withdrawal bleeding occur, but endometrial biopsy specimens are reported to have shown transitional endometrium in three, and fair progestational effect in three cycles. The endometrial changes were interpreted as evidence of effectiveness of the ingested progesterone, although withdrawal bleeding did not occur. We are unable to confirm Bickers' interpretation in this study because withdrawal bleeding was the rule rather than the exception in our series in spite of absence of progestational transformation of the endometrium. Progestational endometrium was found in only one instance after therapy.

Because of lack of sufficient evidence that ingested progesterone is not effective in human subjects, our group has undertaken a study to evaluate the physiologic effects of progesterone administered orally to 25 individuals in whom 64 courses of therapy were studied. Priming with estrogens was employed in 37 courses, and observations were made in 27 courses without

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estrogen priming, in patients in whom the vaginal smears indicated adequate estrogen function (Tables 1 and 2).

METHOD AND MATERIAL

Patients chosen for study were between the ages of 17 and 37. The majority had secondary amenorrhea with persistent proliferative-phase

TABLE 1. ORAL PROGESTERONE (AFTER ESTROGEN PRIMING)

Name	Endometrium before therapy	Oral progesterone therapy	Endometrium after therapy	Pattern of temperature chart	Result
1. H.E.P.	—	100 mg. ×5 (11/1–5)	11/5: E	Anovulatory	Break-through bleeding 11/4
	11/5: E	100 mg. ×5 (11/24–28)	12/3: E	Anovulatory	Withdrawal bleeding 12/1
	12/3: E	100 mg. ×5 (12/23–27)	—	Anovulatory	Withdrawal bleeding 12/31
2. F.N.N.	—	80 mg. ×6 (1/1–6)	—	—	Withdrawal bleeding 1/7
3. M.C.C.	—	80 mg. ×6 (11/20–25)	—	—	Withdrawal bleeding
	—	80 mg. ×6 (12/20–25)	—	—	Withdrawal bleeding
4. O.S.	7/24: E	80 mg. ×2 (8/16–17)	—	Biphasic (8/4)	Break-through bleeding 8/17
5. I.R.	—	80 mg. ×5 (6/20–25)	—	—	Withdrawal bleeding
		80 mg. ×5 (7/15–19)	—	—	Withdrawal bleeding
6. C.S.	—	100 mg. ×6 (2/25–30)	—	—	Withdrawal bleeding 3/7–8
		80 mg. ×5 (3/26–30)	—	—	No bleeding
7. V.H.	—	80 mg. ×5 (3/18–22)	—	Anovulatory	Withdrawal bleeding 3/27
		80 mg. ×5 (4/19–23)	—	Anovulatory	Withdrawal bleeding 4/26
		80 mg. ×5 (5/19–23)	—	? Ovulation	Withdrawal bleeding 4/27
		80 mg. ×5 (6/19–23)	—	Anovulatory	Withdrawal bleeding 6/25
		80 mg. ×6 (12/22–27)	—	Biphasic	Withdrawal bleeding 12/31
8. E.T.S.	11/7: E	100 mg. ×5 (11/16–20)	—	Anovulatory	Withdrawal bleeding 11/23–29
	—	100 mg. ×5 (12/16–20)	—	Anovulatory	Withdrawal bleeding 12/20–25
	—	100 mg. ×5 (1/16–20)	—	—	Withdrawal bleeding
	—	100 mg. ×5 (2/16–20)	—	—	Withdrawal bleeding
9. R.P.C.	—	80 mg. ×5 (6/23–27)	—	Anovulatory	Break-through bleeding 6/24–7/1
10. A.C.H.	—	80 mg. ×3 (3/15–17)	—	—	Break-through bleeding 3/17–20
	—	80 mg. ×5 (4/30–5/4)	—	—	No bleeding
	—	80 mg. ×5 (6/22–26)	—	—	No bleeding
11. M.J.	4/12: E	80 mg. ×5 (5/4–8)	5/12: E	Anovulatory	Withdrawal bleeding 5/12–19
	—	80 mg. ×5 (6/4–8)	—	Anovulatory	Withdrawal bleeding 6/12–17
	—	100 mg. ×6 (10/14–18)	10/24: E	Anovulatory	Withdrawal bleeding 10/24
12. W.R.	—	80 mg. ×5 (5/6–10)	—	Anovulatory	Withdrawal bleeding 5/13–18
13. C.W.	3/25: E	80 mg. ×5 (4/12–15)	4/18: E	—	Withdrawal bleeding 4/18
	4/18: E	80 mg. ×5 (5/8–12)	—	Anovulatory	No bleeding
	—	80 mg. ×7 (Sept. 49)	—	Anovulatory	Withdrawal bleeding
	—	80 mg. ×7 (Oct. 49)	—	Anovulatory	Withdrawal bleeding
	—	80 mg. ×7 (Nov. 49)	—	Anovulatory	Withdrawal bleeding
	—	80 mg. ×7 (Dec. 49)	—	Anovulatory	Withdrawal bleeding
	—	80 mg. ×7 (Jan. 50)	—	Anovulatory	Withdrawal bleeding
14. H.L.N.	2/23: E	80 mg. ×5 (3/13–17)	—	—	Withdrawal bleeding 3/18
15. R.M.	1/19: E	90 mg. ×10 (1/20–29)	1/30: E	Anovulatory	Withdrawal bleeding 1/30

E = Proliferative endometrium.

endometrium; several had menorrhagia with cystic glandular hyperplasia of the endometrium—the study being made during the amenorrheic phase after initial control of the menorrhagia. One patient, aged 19, with primary amenorrhea, presented the typical picture of Turner's syndrome. There was 1 case of primary amenorrhea associated with ovarian dwarfism and 1 case

of pseudocyesis. In 1 patient ovulation was suppressed by large continuous doses of estrogen administered in the therapy of endometriosis; and 1 patient, aged 30, presented the syndrome of "large pale ovaries" with amenorrhea.

TABLE 2. ORAL PROGESTERONE (WITHOUT ESTROGEN PRIMING)

Name	Endometrium before therapy	Oral progesterone therapy	Endometrium after therapy	Pattern of temperature chart	Result
1. O.S.	—	80 mg. ×5 (7/16–20)	7/24: E	Anovulatory	Withdrawal bleeding 7/24
	—	80 mg. ×5 (9/6–10)	—	Anovulatory	Withdrawal bleeding 9/12
	—	80 mg. ×5 (12/19–23)	—	Anovulatory	Withdrawal bleeding 12/24
2. E.C.W.	—	100 mg. ×5 (12/1–5)	12/17: E	Anovulatory	No bleeding
3. H.W.	—	80 mg. ×7½ (8/1–7)	—	Anovulatory	Withdrawal bleeding 8/9
	—	80 mg. ×7½ (8/30–9/6)	9/7: E	Anovulatory	Withdrawal bleeding 9/7
4. L.P.	—	100 mg. ×7 (10/25–31)	—	Anovulatory	Withdrawal bleeding 11/1
	—	100 mg. ×5 (11/22–26)	—	Anovulatory	Withdrawal bleeding 11/29
	—	100 mg. ×5 (12/20–24)	—	Anovulatory	Withdrawal bleeding 12/26
	—	100 mg. ×5 (1/18–22)	1/23: P	Anovulatory	Withdrawal bleeding 1/23
5. V.H.	—	80 mg. ×5 (7/17–21)	—	Anovulatory	Withdrawal bleeding 7/25
	—	80 mg. ×5 (8/15–19)	—	Anovulatory	Withdrawal bleeding 8/23
	—	80 mg. ×5 (11/24–28)	—	Anovulatory	Withdrawal bleeding 12/2
	—	100 mg. ×5 (1/20–24)	1/28: E	Anovulatory	Withdrawal bleeding 1/28
6. N.B.	—	80 mg. ×5 (2/8–12)	2/14: E+	Anovulatory	Withdrawal bleeding 2/10
7. K.V.	—	100 mg. ×5 (1/14–18)	—	—	Break-through bleeding 1/15
8. A.C.H.	—	80 mg. ×7½ (8/7–14)	—	—	Withdrawal bleeding 8/14
	—	100 mg. ×3 (9/3–5)	—	—	Break-through bleeding 9/5
	—	100 mg. ×7 (10/9–15)	—	—	Withdrawal bleeding 10/15
	—	100 mg. ×5 (11/3–7)	—	—	No bleeding
9. M.J.		80 mg. ×5 (6/28–7/2)	—	Anovulatory	Withdrawal spotting 7/3
10. M.W.	2/9: E+	100 mg. ×5 (2/10–14)	2/16: E	—	Withdrawal bleeding 2/18
11. T.J.S.	1/28: E+	100 mg. ×5 (1/29–2/2)	2/11: E	—	Withdrawal bleeding 2/11
12. S.T.	2/13: E—	200 mg. ×5 (2/14–19)	2/19: E	Anovulatory	Withdrawal bleeding 2/20
13. M.S.	—	100 mg. ×5 (2/17–21)	—	—	Withdrawal bleeding
14. J.E.H.	—	100 mg. ×5 (2/5–9)	—	—	Withdrawal bleeding 2/16
15. R.M.	1/19: E	200 mg. ×5 (2/20–24)	3/10: P*	Biphasic	Delayed bleeding-menses 3/10

E = Proliferative endometrium.
E− = Atrophic endometrium.
E+ = Cystic glandular hyperplasia.
P = Secretory changes present.
P* = Ovulatory menstrual endometrium.

The criteria of effectiveness of orally ingested progesterone in this study were:

1. The induction of "progesterone withdrawal bleeding" in amenorrheic patients, usually within two to five days after cessation of therapy, in the absence of spontaneous ovulation.

2. The absence of endogenous progesterone (from a corpus luteum) as evidenced by an anovulatory pattern of the basal temperature record.

3. Observation of changes in the endometrium by aspiration curettage before and after therapy, whenever feasible. The first day of withdrawal bleeding was chosen for this procedure.

4. In 4 instances, chosen at random, urinary pregnanediol determina-

Date	Day of cycle	Basal temperature 97° .2 .4 .6 .8 98° .2 .4 .6 .8	Menses	Therapy
2– 9–50	11			
10	12			
11	13			
12	14			
13	15			
14	16			
15	17			
16	18			
17	19			
18	20			
19	21			
20	22			∇=E (2–22–50)
21	23			Oral progesterone 200 mg.
22	24			Oral progesterone 200 mg.
23	25			Oral progesterone 200 mg.
24	26			Oral progesterone 200 mg.
25	27			Oral progesterone 200 mg.
26	28			
27	29			
28	30			
3– 1–50	31			
2	32			
3	33			
4	34			
5	35			
6	36			
7	37			
8	38			
9	39			
10	1		X	∇=*P* (Ovulatory menstrual endometrium)

FIG. 1. Delayed menstruation after oral progesterone therapy. This bleeding cannot be attributed to progesterone, since spontaneous ovulation occurred and bleeding took place thirteen days thereafter. (∇=suction curettage; E=proliferative endometrium; P=ovulatory menstrual endometrium.)

tions over a 24-hour period before and after therapy were employed.

The total dose of progesterone was from 400 to 700 mg. orally in divided dosage over a period of five to seven days (80 to 100 mg. daily) (*see* tables). In 2 instances 200 mg. was given daily for five days up to a total dose of 1000 mg. in an unsuccessful attempt to produce a progestational endometrium. These 2 patients did not receive estrogen priming since they had an estrogenic vaginal smear. In 1 of these patients spontaneous ovulation

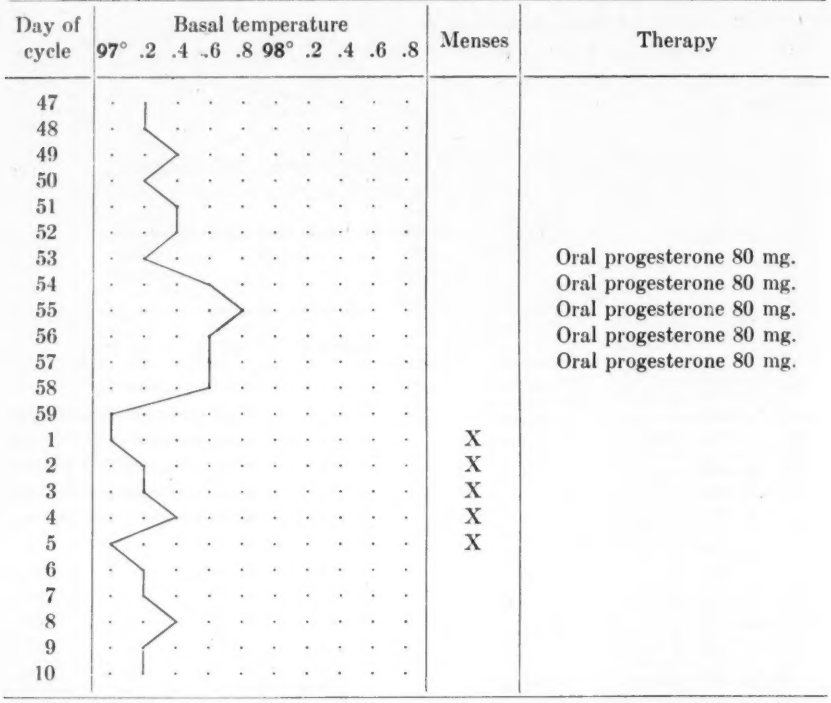

FIG. 2. Note rise in basal temperature following oral progesterone therapy.

occurred so, of course, endometrial changes could not be credited to oral progesterone therapy (Fig. 1). In another instance, as previously mentioned, the estrogen therapy was continuous in relatively large dosage, and oral progesterone therapy was superimposed for five days each month to produce a period of withdrawal bleeding with shedding of the endometrium in order to prevent excessive bleeding of prolonged estrogen stimulation (Table 1, Case 8).

The progesterone used in this study was prepared in uncoated, compressed tablets containing 20 mg. of progesterone. The estrogen used, for the most part, was in the form of gelatin capsules, each containing 4000 I.U. of natural estrogens in oil.

RESULTS

Withdrawal bleeding occurred in 90.2 per cent (55 of 61 instances) of the total courses of therapy studied, usually within seventy-two hours after completion of therapy. Bleeding lasted three to five days and was equal in amount to a normal menstrual period. The frequency with which with-

drawal bleeding occurred after oral progesterone therapy, with or without estrogen priming, provides adequate evidence of the absorbability and utilization of ingested progesterone. The results were essentially uniform whether or not the patients received estrogen priming prior to progesterone therapy. Withdrawal bleeding occurred in 88.6 per cent (31 of 35 courses) of estrogen primed cases, and in 92.3 per cent (24 of 26 courses) without estrogen priming. This tends to obviate the consideration that this may be estrogen withdrawal bleeding in the estrogen primed cases because, if this

TABLE 3

	Courses of therapy*	Withdrawal bleeding	No result	Per cent effectiveness
Total	61	55	6	90.2
Oral progesterone (with estrogen priming)	35	31	4	88.6
Oral progesterone (without estrogen priming)	26	24	2	92.3

* Actually 64 courses of therapy were administered. The results in 3 are omitted, since spontaneous ovulation occurred and bleeding could not be credited to the administered progesterone.

were true, a higher incidence of bleeding would be expected in the primed group. If ovulation took place before or during the period of oral progesterone therapy with this dosage, we observed that true menstruation usually occurred approximately fourteen days later regardless of the course of therapy (Fig. 1). It must be remembered that all are cases of amenorrhea, and that basal temperature charts (with three exceptions, discussed separately) indicated the typically monophasic curve of ovulatory failure (Fig. 2). Excluded from our statistical data are the 3 patients in whom spontaneous ovulation occurred, since neither withdrawal bleeding nor endometrial changes could be properly credited to the oral progesterone therapy (Table 3).

In 6 instances the term "break-through bleeding" was used to indicate that uterine bleeding started after the beginning, but before completion, of the course of oral progesterone therapy. Two examples of this occurred in the series without estrogen priming and 4 in the estrogen primed series. In 1 patient (Table 1, Case 4) the basal temperature chart indicated that ovulation occurred on the ninth day of estrogen therapy. Estrogen therapy was continued for eleven more days, and then oral progesterone 80 mg. daily was given. Bleeding occurred on the third day after initiation of

progesterone therapy—the fourteenth day after ovulation. Occasionally, during estrogen therapy alone, the basal temperature graph indicated that this patient ovulated, and true menstruation occurred fourteen days later; however, on repeated occasions, during a prolonged anovulatory amenorrheic phase, withdrawal bleeding was induced from a proliferative endometrium (Table 2, Case 1; and Table 1, Case 4) four days following oral progesterone therapy, 80 mg. daily for five days. This is 1 of only 3 instances in which evidence of ovulation appeared. In another patient (Table 1, Case 7) bleeding began four days after therapy—the thirteenth day after ovulation. This same patient was given progesterone orally without estrogen priming in the preceding and subsequent cycle, and on these occasions, with a typically anovulatory basal temperature graph, withdrawal bleeding was induced four and two days respectively, after completion of five daily doses of 80 mg. of progesterone by ingestion (Table 2, Case 1).

Another example of break-through bleeding (Table 1, Case 9) in the estrogen primed series was that of a patient 34 years of age who had only three "menstrual periods" in the preceding thirteen years. All three of these periods were induced by progesterone administration, one of which, included in this series, occurred following twenty days of oral estrogen therapy succeeded by progesterone orally, 80 mg. daily. Bleeding began on the second day of progesterone therapy. The basal temperature record did not reveal the biphasic graph of ovulation. Incidentally, after four years of study from the standpoint of infertility (primary ovulatory failure), this patient conceived and delivered a 7 lb. 3 oz. infant.

In the patients studied by correlation of the basal temperature record with therapy and withdrawal bleeding, we have observed a prolonged low level of the temperature graph during the periods of prolonged amenorrhea and failure of ovulation. In the majority of these women, the day after beginning oral progesterone therapy, there was a consistent and unquestionable rise in basal temperature which continued at the elevated level throughout the course of therapy (Fig. 2). A decline in the basal temperature occurred about seventy-two hours after completion of therapy, or coincident with the onset of withdrawal bleeding. This is cited as another possible evidence of the physiologic effectiveness of ingested progesterone. In a study on surgically castrated women, Israel (4) has shown that 60 to 80 mg. of pregneninolone daily raised the body temperature 0.4° to 0.6° F.

A case study. Figure 2 is the basal temperature record of a 25-year-old unmarried patient with functional amenorrhea (Table 1, Case 15). The temperature curve throughout two successive courses of therapy was observed. Aspiration curettage after amenorrhea for seven months revealed a proliferative phase endometrium. Progesterone was administered orally in doses of 30 mg. (in combination with oral estrogens) three times

daily for a total of 900 mg. in ten days. In this instance, the temperature did not show the usual rise during the period of therapy. One day after completion of this course of therapy, however, withdrawal bleeding began and lasted four days. Aspiration curettage on the first day of withdrawal bleeding again revealed a proliferative phase endometrium, but this time with some evidence of occasional secretory changes. Hence, correlation of the endometrial biopsy findings and the basal temperature record indicates that withdrawal bleeding from a proliferative endometrium began one day after completion of oral progesterone therapy in absence of ovulation. The subsequent cycle, however, illustrates an entirely different phenomenon (Table 2, Case 15). Aspiration curettage on the twenty-second day of the cycle, again revealed proliferative phase endometrium. On each of the five following days the patient was given 200 mg. of progesterone by ingestion (a total of 1000 mg.). There was no rise in basal temperature during the days of this therapy, but on the second day after completion of therapy, the twenty-ninth day of the cycle, there was a definite rise to 98.2° F., which was maintained for the following thirteen days. Withdrawal bleeding did not immediately follow this course of therapy, since spontaneous ovulation had intervened and menses occurred as would be expected about thirteen or fourteen days later. Aspiration curettage on the first day of bleeding revealed ovulatory menstrual endometrium.

Several conclusions were drawn from this type of response, *i.e.*, withdrawal bleeding can be induced, in the absence of ovulation, with a total dose of orally ingested progesterone which is insufficient to produce a secretory endometrium; if ovulation occurs during or shortly after the period of administration of progesterone orally, withdrawal bleeding will be delayed, occurring about thirteen to fourteen days after spontaneous ovulation; and that proper evaluation of the presence or absence of withdrawal bleeding or of endometrial changes, as related to progesterone therapy, cannot be made without concurrent consideration of some index of ovulatory function such as the basal temperature record.

Aspiration curettage. Endometrial scrapings were obtained in 16 instances on the first day of withdrawal bleeding following oral progesterone therapy. Six of these were in the estrogen primed group, and 10 in the group without estrogen priming. In only 1 instance without ovulation was a progestational endometrium obtained (Table 2, Case 4). The basal temperature graph in this case revealed the typically monophasic curve of an anovulatory cycle, with an elevation in temperature during the five days of oral progesterone therapy only. Withdrawal bleeding began on the day after completion of progesterone therapy—the sixth day after the first elevation of the basal temperature, which occurred about twenty-four hours after beginning oral progesterone. Had this endometrial change been

due to ovulation, the withdrawal bleeding would not have occurred and normal menses would have been delayed until fourteen days after ovulation. The three immediately preceding cycles in this same patient were episodes of oral progesterone withdrawal bleeding and the accompanying basal temperature charts showed an anovulatory pattern.

An attempt to produce a progestational endometrium with a larger dose of oral progesterone was made on 2 patients. In 1 (already discussed in detail), the secretory endometrium could not be credited to oral progesterone therapy, since ovulation occurred. The other (Table 2, Case 12) was a 26-year-old patient with secondary amenorrhea and a monophasic temperature graph. The vaginal smear was interpreted as mature and muco-cornified (androgenic smear) (5). Aspiration curettage before the course of therapy revealed cystic glandular hyperplasia of the endometrium. The patient was given 200 mg. of oral progesterone daily for five days. On the last day of therapy, aspiration curettage revealed a proliferative phase endometrium with no secretory changes. The basal temperature graph was that of an anovulatory phase. Withdrawal bleeding began within thirty-six hours after completion of therapy.

Allen (6) previously observed a measurable increase in urinary excretion of a conjugated steroid (presumably sodium pregnanediol glucuronide) during and after oral administration of progesterone (as well as anhydrohydroxyprogesterone). He also observed that the maximum levels of excretion following anhydrohydroxyprogesterone and progesterone in equivalent dose were about the same; however, the excretion of pregnanediol following progesterone was much more rapid than that following anhydrohydroxyprogesterone. Since withdrawal bleeding failed to occur following oral administration of 200 mg. of progesterone daily for six days (in a single case study), Allen assumed the oral progesterone to be inactive and suggested that, if this one observation be typical, it indicated that progesterone may be converted into pregnanediol too rapidly to be physiologically effective when ingested. Our observations confirm those of Allen as regards increased urinary excretion of pregnanediol after oral progesterone administration. Since the results observed in this study also present evidence of physiologic action of ingested progesterone, it might be speculated that progesterone ingested actually may be more readily absorbed and utilized, hence is physiologically less effective than anhydrohydroxyprogesterone. This preliminary report will comprise the basis for presentation of a more complete comparative study which is now in progress.

The excretion of pregnanediol in a 24-hour urine specimen was determined before and after a course of oral progesterone therapy in 4 patients. The procedures employed were hydrolysis, extraction and preparation of

the neutral fraction, following the method described by Pincus (7); and after a Girard separation of ketonic and nonketonic steroids, the purification and colorimetric assay of pregnanediol as described by Talbot, Berman, MacLachlan and Wolfe (8). A significant increase in the urinary content of pregnanediol after oral administration of progesterone may be regarded as further evidence of absorption from the normal gastrointestinal tract (Table 4).

TABLE 4. URINARY EXCRETION OF PREGNANEDIOL BEFORE AND AFTER ORAL PROGESTERONE THERAPY

Name	Urinary pregnanediol (24 hr.)	Oral progesterone therapy	Urinary pregnanediol (24 hr.)	Basal temperature pattern
H.N.*	3/12: 0.43 mg.	80 mg. ×5d 3/13–17	3/18: 5.40 mg.	Anovulatory
N.B.	2/8 : 0.99 mg.	80 mg. ×5d 2/8–12	2/14: 8.97 mg.	Anovulatory
S.T.	2/13: 2.44 mg.	200 mg. ×5d 2/14–19	2/18: 30.9 mg.	Anovulatory
S.H.	2/13: 2.63 mg.	300 mg. ×3d 2/14–16	2/16: 20.8 mg.	Anovulatory

* Estrogen primed.

SUMMARY AND CONCLUSIONS

1. Observations were made on 25 amenorrheic patients during 64 courses of therapy with oral administration of progesterone. The usual course of therapy was 80 to 100 mg. of progesterone daily in divided doses over a period of five days. Estrogen priming for fifteen to twenty days was employed in 37 courses of therapy in 15 patients. One of the prerequisites for the administration of progesterone alone to patients with amenorrhea was their adequate intrinsic production of estrogens as demonstrated by estrogenic vaginal smears.

2. Withdrawal bleeding occurred about twenty-four to seventy-two hours after completion of therapy in 90.2 per cent of cases (88.6 per cent in the estrogen primed series, and 92.3 per cent in the series with oral progesterone alone).

3. The ingestion of a total dose of 400 to 500 mg. of progesterone in three to five days was insufficient to cause secretory changes in the endometrium except in 1 instance.

4. Elevation of the basal temperature during the period of administration of oral progesterone in the absence of spontaneous ovulation occurred with significant frequency.

5. Absence of ovulation was evidenced by a typical monophasic graph of the daily basal temperature. In a few instances spontaneous ovulation occurred and bleeding was delayed until thirteen or fourteen days after

the rise in basal temperature. Of course, in these instances, neither bleeding nor secretory changes in the endometrium could be credited to therapy.

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A CASE OF PHEOCHROMOCYTOMA: DIAGNOSIS BY THE BENZODIOXANE TEST, URINARY HORMONE STUDIES, AND NOR-EPINEPHRINE ASSAY OF THE TUMOR

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MORE cases of pheochromocytoma are being seen today because of the current interest in the etiology and pathogenesis of hypertension. Many of these patients present sustained hypertension rather than the classic, paroxysmal type. In a recent review of 51 cases, Green (1) found that 14 subjects showed intermittent and 37 chronic hypertension. With the development of the Benzodioxane test by Goldenberg *et al.* (2), a method for the screening of hypertensive patients for the presence of this tumor became available.

This communication is concerned with a patient, observed in this hospital for over two years, in whom the disease simulated renal hypertension. The diagnosis of pheochromocytoma was made by means of the Benzodioxane test and was followed by successful removal of the tumor. A pharmacologic study of the tumor was made, which gave evidence of the presence of nor-epinephrine.

CASE REPORT

H.D.M., #37319, a 12-year-old, colored male, was *first admitted* November 25, 1946, in a semi-comatose state. According to his parents he had been well until four days before admission when he complained of a severe headache and repeated attacks of vomiting. Immediately before admission he had several clonic convulsions. When first examined, he was unconscious, the blood pressure was 180/80, the pulse rate 130; there were no abnormal neurologic findings, no choked discs, and the spinal fluid was normal. The coma became deeper; a pneumoencephalogram taken at this time did not reveal anything abnormal. Following this procedure the patient went into a state of shock. Plasma and whole blood were administered and within a few days he became conscious and rational. On discharge the blood pressure was 160/80.

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Second admission. On February 2, 1947, he was again admitted, because of clonic and tonic convulsions. He had been well since the previous admission except for abdominal pain, nausea, vomiting and some decrease in visual acuity. When seen he was conscious and rational but quite restless; the blood pressure was 208/158, the pulse rate 136, and the oral temperature 98.6° F. There were hemorrhages and exudates in both retinae, choking of the left disc, and blurring of the right. The heart was enlarged to the left anterior axillary line and the point of maximum impulse was in the sixth intercostal space. Neurologic examination revealed a slight muscular weakness of the left extremities, a slight incoordination of skilled movements of the left hand, and a left Babinski response. The spinal fluid on lumbar puncture had a pressure of 600 mm. of water but was otherwise normal. A ventriculogram showed no sign of intracranial disease. Evidence of myocardial damage was disclosed by the electrocardiograph but there was no axis deviation. An albuminuria and a low specific gravity were repeatedly observed on urinalysis. The Fishberg concentration test resulted in a maximum specific gravity of 1.008, and a phenolsulphonphthalein test showed an excretion of 15 per cent of the dye in two hours. An intravenous pyelogram and an Addis count showed no abnormalities.

The condition of the patient improved after treatment consisting of sedation and a low salt, high protein diet. The blood pressure dropped to 180/140 and the spinal fluid pressure gradually fell to 90 mm. of water. He was discharged on March 5, 1947. In the ensuing months he was seen in the outpatient clinic, where the blood pressure usually ranged between 150/100 and 180/120; occasionally it rose to as much as 200/120, at which times the patient complained of nausea.

A *third admission* occurred on August 18, 1947, when the chief complaint was nausea and vomiting. The physical and ophthalmologic examinations resulted in essentially the same findings as on the second admission except that there were no abnormal neurologic signs. The blood pressure was 210/160. An Addis count at this time revealed 880,000 casts, 1,200,000 white blood cells, and 9,600,000 red blood cells in a twelve-hour specimen of urine. The blood NPN was 50 mg. per cent. A diagnosis was made of chronic glomerulonephritis with hypertensive cardiovascular disease. The blood pressure was 210/120 and the pulse rate 80 on discharge. He was seen in the outpatient clinic on October 22, 1947, when the blood pressure was 170/120 and the eyeground findings remained unchanged. He then ceased visiting the outpatient clinic.

The *final hospital admission* took place on April 9, 1949, more than two years and four months after he was first seen. He was admitted in a stuporous state with a spastic paralysis of the left extremities, which suddenly took place after a nightmare. On examination, a left Babinski response, a left facial paralysis, deviation of the eyes to the left, and very cold extremities were noted. The blood pressure was 240/180, the pulse rate was 160 per minute, and the oral temperature 98.8° F. There were blurred discs, fresh linear hemorrhages, and old exudates in the fundi. The heart was enlarged to the left anterior axillary line. Immediate therapy consisted of large doses of phenobarbital sodium, magnesium sulfate, and an intravenous infusion of 500 ml. of 5 per cent glucose in water containing 30 ml. of ethanol. The blood pressure fell to 190/145, but within twelve hours it returned to 240/180.

The *Benzodioxane test* (2) was performed and the results were positive. The patient received 20 mg. of Benzodioxane[1] by intravenous infusion; there followed a prompt drop in the blood pressure to 125/90 with a return to the original level one hour later (Fig. 1 and Table 1). On April 15, 1949, a *Dibenamine test* was performed with similar results.

[1] 2-(1-Piperidylmethyl)-1,4-benzodioxan (Merck).

The patient was given 400 mg. of Dibenamine[2] in 500 ml. of 5 per cent glucose in water over a period of two hours. The blood pressure fell from 210/160 to 110/70 within forty minutes, remaining at approximately this level for eleven hours, after which it gradually rose to 230/130, forty-eight hours after the infusion was begun. Spear and Griswold (3) have used Dibenamine in pheochromocytoma.

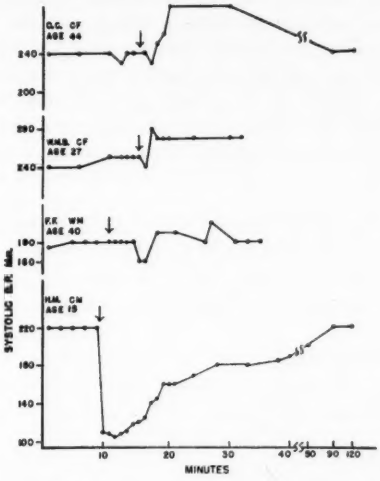

FIG. 1. Examples of systolic blood pressure responses to the Benzodioxane test. The 3 upper lines show negative responses in O.C. (malignant hypertension), in W.M.B. (renal hypertension), and in F.F. (essential hypertension). The final patient, H.M., is the subject of this report. At the arrows, Benzodioxane (10 mg. per square meter of body surface) was injected intravenously.

Intravenous pyelograms showed neither renal displacement nor distortion, but revealed a small area of increased density medial to the superior calyx of the right kidney,

TABLE 1. BLOOD PRESSURE RESPONSES TO THE BENZODIOXANE TEST IN A CASE OF PHEOCHROMOCYTOMA

Date	Blood pressure in mm. of Hg			
	Initial	After Benzodioxane injection		
		3 minutes	15 minutes	60 minutes
4/11/49	220/170	125/90	190/140	230/160
4/11/49	220/160	105/70	160/125	210/150
	(122)**	(144)	(136)	(122)
8/ 4/49*	120/80	140/95	130/80	

* Fourteen weeks after removal of tumor.

** Pulse rate.

[2] N,N-Dibenzyl-β-chloroethylamine hydrochloride (Smith, Kline and French).

which was interpreted to be calcification within the kidney. Electrocardiographic findings indicated myocardial damage and an active myocardial process. A teleoroentgenogram of the heart revealed enlargement of the left ventricle; the C.T. ratio was 11.4/27.5 and the length of the left ventricular segment was 13.3 cm. Laboratory findings were as follows: The spinal fluid had a pressure of 380 mm. of water. It showed a trace of globulin and a first-zone gold curve but otherwise it was normal. The fasting blood sugar at one time was 144 mg. per cent and the oral glucose tolerance curve was elevated (Table 2). The blood chlorides were 110 mEq. per liter and the blood NPN was 34 mg. per cent. A 24-hour urine specimen was collected on April 4, 1949, for urinary hormone studies (Table 3). There were 4,800,000 red blood cells per cu. mm. and 14 Gm. per cent hemoglobin. The white blood count was 12,400 per cu. mm. with 16 per cent lymphocytes, 2 per cent monocytes, 76 per cent segmented forms, 2 per cent stab cells, and 3 per cent juvenile cells.

TABLE 2. GLUCOSE TOLERANCE TESTS BEFORE AND AFTER REMOVAL OF TUMOR
(No Glycosuria at Either Time.)

Date	Blood Sugar (mg. per cent)					
	Fasting	½ hr.	1 hr.	2 hrs.	3 hrs.	5 hrs.
4/20/49	120	180	229	168	156	
5/12/49	117	144	114	117	100	96

TABLE 3. URINARY HORMONE STUDIES BEFORE AND AFTER REMOVAL OF A PHEOCHROMOCYTOMA

Date	Cortin mg./24 hrs.	17-Ketosteroids mg./24 hrs.			Gonadotropin units/24 hrs.	Creatinine Gm./24 hrs.
		Alpha	Beta	Total		
4/14/49	0.8	6.5	0.3	6.8	690.	1.04
4/25/49	Surgical removal					
5/12/49	0.1	9.5	0.3	9.8		0.784
8/ 4/49	0.9	6.4	0.4	6.8	720.	0.684

The patient was subjected to surgery on April 25, 1949. In addition to routine preoperative medication, he was given 10 ml. of adrenal cortical extract intramuscularly. Under endotracheal ether anesthesia an upper transverse abdominal incision was made. On opening the peritoneal cavity the left kidney and adrenal gland were identified and found to be normal to inspection and palpation. No tumor masses were found along the para-aortic areas or at the bifurcation of the aorta, but on examining the right kidney a tumor mass was found at its superior pole. This mass was removed without great difficulty.

As soon as the tumor was removed, 0.12 ml. of epinephrine solution was given intravenously. To an infusion of 5 per cent glucose in saline already started, 4 mg. of epinephrine and 10 ml. of adrenal cortical extract were added. The blood pressure was 90/60 at the end of the operation.

The patient was constantly attended by a member of the house staff or a medical student for the next forty-eight hours, during which period the blood pressure pulse, and respiration were observed every five to fifteen minutes. The blood pressure was maintained at approximately 110/70 with the aid of intravenous clyses of 5 per cent glucose containing 4 mg. per liter of epinephrine. As the blood pressure was found to vary with the rate of infusion, eight hours after surgery this therapy was changed to Neosynephrine in a dose of from 2 to 4 mg. subcutaneously every sixty or ninety minutes, which was found to maintain blood pressure satisfactorily. Occasionally ephedrine, 50 mg. every two or three hours, was given subcutaneously. The sympathomimetic drugs were gradually withdrawn between forty-eight and sixty hours after surgery. The postoperative course was complicated by a diffuse, patchy atelectasis of the entire right lung; the patient was bronchoscoped and the infection was held in check with 100,000 units of penicillin intramuscularly every three hours. The patient was afebrile and asymptomatic by the eighth postoperative day.

On May 12, 1949, the glucose tolerance was found to be normal (Table 2). Urinary hormone studies were repeated (Table 3). An x-ray plate of the chest showed clear lung fields but the heart shadow was no smaller than that observed in the preoperative film. He was discharged on May 13, 1949, feeling well but unable to walk because of the left hemiplegia.

The patient was readmitted on August 1, 1949, for follow-up studies. He was able to walk and was completely asymptomatic except for a slight limp. The blood pressure was 122/80 and the pulse rate 88. Except for evidence of old exudates in the retinae, both eyegrounds were normal. There was some muscular weakness and slightly hyperactive reflexes in the left lower extremity; the rest of the examination revealed nothing abnormal. An electrocardiogram was interpreted as still showing evidence of myocardial damage, with some reversion towards normal. A teleoroentgenogram revealed some decrease in the heart size; the C.T. ratio was 10.6/27.2 and the length of the left ventricular segment was 11.6 cm. There was a negligible blood pressure response to the Benzodioxane test (Table 1). An Addis count showed 700,000 red blood cells, 300,000 white blood cells, and no casts in a 12-hour specimen of urine. The fasting blood sugar was 109 mg. per cent and the blood chlorides were 140 mEq. per liter. Urinary hormone studies were repeated (Table 3).

The patient was seen again on October 30, 1949, in the outpatient clinic. His vision was normal but he walked with a slight limp. He had sparse pubic hair and underdeveloped genitalia which had been noted previously.

PATHOLOGIC REPORT

(by Dr. L. H. Findley and Dr. J. F. A. McManus of the Department of Pathology)

"The specimen consists of an oval mass of moderately firm, reddish-gray tissue and at either end of the two poles, some yellowish tissue grossly resembling adrenal gland. The mass weighs 45.4 Gm. and is 6 by 4.5 cm. in size. On cut section there is found a rather firm, gray, central portion measuring 1.5 cm. in diameter. Surrounding this there is a zone of bright red tissue with streaks of gray radiating out through it from the central mass, the hemorrhagic zone averaging 1 cm. in width. Outside of this and forming the main peripheral mass of the tumor is some grayish-pink, somewhat softer tissue averaging 1.5 cm. in thickness. The measurements of the tumor, not including the yellow adrenal tissue attached, are 4.5 by 4.5 cm. (Fig. 2)."

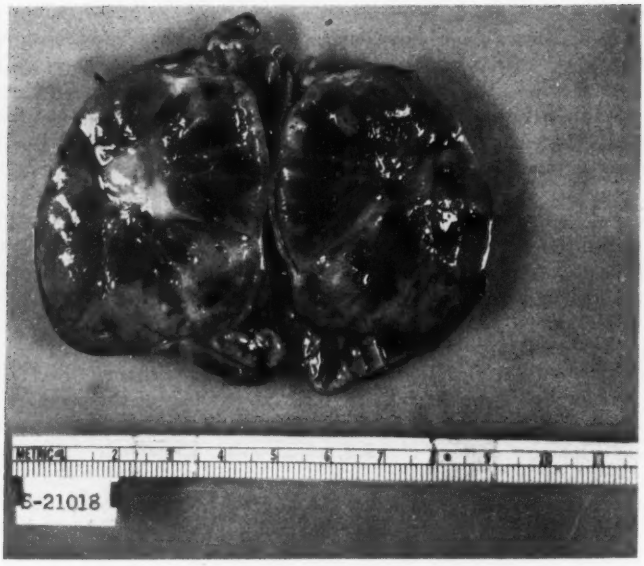

FIG. 2. Gross appearance of the tumor.

"Microscopically, the tumor is made up of large, polyhedral, epithelial-like, lightly basophilic cells. They are arranged in packets bounded by light fibrous septa and occasionally something resembling acini can be made out. The cells are generally large but vary in size and shape. Giant forms are also seen. The nuclei vary considerably in shape but no mitotic figures are seen. Pigmentation of the tumor cells is not very striking. Diagnosis: *pheochromocytoma* (Fig. 3)."

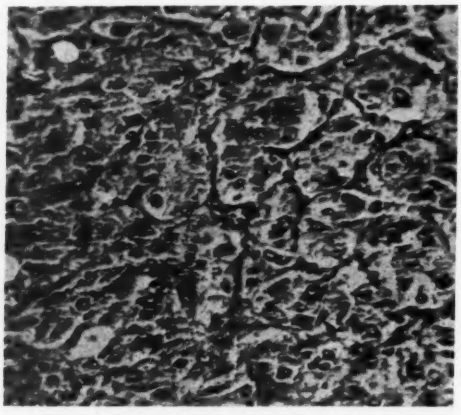

FIG. 3. Photomicrograph of the tumor. Hematoxylin-eosin. ×200.

PHARMACOLOGIC STUDIES ON THE TUMOR EXTRACT

Recent pharmacologic and chemical investigations of extracts of pheochromocytomas and normal adrenal glands have shown that these tissues contain both epinephrine and nor-epinephrine (arterenol). Holton (4) found that 3 such tumors, examined by pharmacologic methods, contained from 0.32 to 0.37 mg. of epinephrine and 6.3 to 11 mg. of nor-epinephrine per gram of tissue. These findings were confirmed by enzymatic and chromatographic techniques. Pitcairn and Youmans (5) determined the effect of an aqueous extract of a pheochromocytoma on the blood pressure of dogs before and after Dibenamine and showed that this extract possessed a potent pressor action which was not blocked by full adrenolytic doses of Dibenamine. Using the method of paper chromatography, the epinephrine fraction of 3 chromaffin tissue tumors were found by Goldenberg *et al.* (6) to contain 47, 12, and 10 per cent epinephrine and 53, 88, and 90 per cent nor-epinephrine, respectively. Examination of U.S.P. Epinephrine Solution by this same method revealed a nor-epinephrine content of 0 to 36 per cent. In a recent note, Graham (7) has stated that 10.6 mg. of *l*-nor-epinephrine and 2.7 mg. per Gm. of *l*-epinephrine equivalents were found by biologic assay in an adrenal medullary tumor. Finally, Beyer *et al.* (8) have assayed 2 tumors for pressor activity before and after Dibenamine and have reported an activity of 10.3 mg. per Gm. and 11.8 mg. per Gm., respectively, in terms of epinephrine. In the first case about 50 per cent of the pressor principle was nor-epinephrine, whereas in the second it appeared to be 90 per cent.

The chromatographic demonstration of nor-epinephrine in extracts of U.S.P. Epinephrine Solution (6) was followed by the isolation by Tullar (9) of crystalline nor-epinephrine from a sample of natural U.S.P. Epinephrine. Auerbach and Angell (10) have described a colorimetric method for the assay of nor-epinephrine in the presence of epinephrine and have reported that U.S.P. Epinephrine contains 10.5 to 18.5 per cent nor-epinephrine as determined by this method. Using other chemical techniques, von Euler and Hamberg (11) found that the adrenal glands of cattle contained 0.68 to 2.68 mg. of nor-epinephrine and 1.8 to 11.3 mg. of epinephrine per gram of fresh tissue. Shümann (12) has given evidence of the presence of nor-epinephrine in the adrenals of pigs. Bülbring and Burn (13), using the nictitating membrane and other responses, have concluded that stimulation of the splanchnic nerve in the spinal cat is followed by the liberation of both epinephrine and nor-epinephrine. These investigators have also adduced evidence that the adrenals of dogs contain both compounds.

EXPERIMENTAL

Biologic assay. About one-half of the tumor (18.5 grams) remained in a refrigerator for three days after its removal in 95 per cent ethanol containing 3 per cent acetic acid; it was then ground finely and extracted for three days in the cold with this solvent. This solution was assayed for pressor activity on the next day and frequently thereafter on atropinized dogs under Dial-Urethane anesthesia. Comparisons were made with the activity of Adrenalin Chloride (Parke-Davis), epinephrine (Eastman),

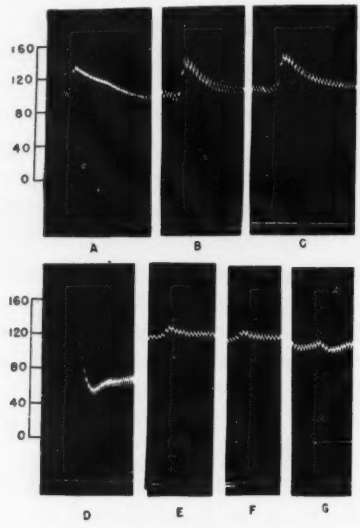

FIG. 4. Blood pressure responses in atropinized dog. A) 0.01 mg. of epinephrine (Parke-Davis). B) 0.005 mg. of *d,l*-arterenol. C) the tumor extract, representing .0045 Gm. of original tumor. Dibenamine, 25 mg. per Kg. given between C and D. D) repetition of A. E) repetition of B. F) repetition of C. G) a mixture of .0045 mg. of arterenol and .00045 mg. of epinephrine.

d,l-arterenol (Winthrop-Stearns), and mixtures of epinephrine and arterenol. Observations were made of the blood pressure changes both before and after the administration of Dibenamine. Based on a study of numerous injections in 6 dogs, it was concluded that one gram of the tumor possessed an average pressor activity equivalent to 0.86 mg. of *d,l*-arterenol. A portion of the extract was made alkaline, allowed to stand for three days in the cold, reacidified, and assayed. This fraction was found to be purely depressor.

After completely adrenolytic doses of Dibenamine, the tumor extract still produced a rise in blood pressure although the response was greatly

reduced in magnitude; a mixture of one part of epinephrine and ten parts of arterenol gave a slight depressor response after Dibenamine (Fig. 4). It was concluded that over 90 per cent of the pressor activity of the tumor was due to nor-epinephrine.

Paper chromatography. The method of Goldenberg *et al.* (6) was applied to aqueous and to alcoholic solutions of the tumor extract. Paper chromatograms were developed and compared with those of solutions of epinephrine, Adrenalin Chloride, and *d,l*-arterenol. Spots were obtained typical of both nor-epinephrine and epinephrine. An average value corresponding to 1.04 mg. of *d,l*-arterenol per gram of tissue was found. The amount of epinephrine present was so small that quantitative estimation was not deemed feasible.

Colorimetric determination of the nor-epinephrine content of the tumor by the method of Auerbach and Angell (10) was attempted. As extremely high values, obviously in error, were obtained these experiments were abandoned.

DISCUSSION

The value of the Benzodioxane test both in the screening of hypertensive patients for pheochromocytoma and in assuring the diagnosis with a fair degree of certainty is well illustrated by this case. Goldenberg *et al.* (2) have performed the test on 58 patients with various types of hypertension and found a positive response in 4 patients, all of whom proved to have pheochromocytomas. Grimson *et al.* (14) found 1 positive response (in a case of the tumor) out of 61 cases of hypertension tested. We have performed the test in 30 cases of sustained hypertension, with negative results in all but the case described here. Examples of the responses are shown in Figure 1. As yet no one has failed to find the tumor after a positive response to the test has been obtained. Taliaferro (15) has reported a case of renal hypertension in which the results of a Benzodioxane test were supposedly positive; but on three repetitions of the test, the reactions became progressively negative. We have found the test to be quite reproducible in this patient, and found a reversion to a negative response after successful removal of the tumor (Table 1). Confirmatory evidence for the diagnosis was obtained in the striking drop in blood pressure after the administration of Dibenamine. If a positive Benzodioxane test is noted in a patient and localization of the tumor cannot be shown by intravenous pyelography, we believe it advisable to repeat the test several times and also to note the response to Dibenamine. If positive responses are obtained consistently, exploration is justified. It must be pointed out, however, that the Benzodioxane test is not entirely innocuous. Pressor responses have been reported (16) and we have observed a rise in blood pressure from 180/120 to

a peak of 260/130 three minutes after injection of the drug in a case of essential hypertension.

As for the operative aspects of this case, when no localization of the tumor could be made by pyelography and perirenal air insufflation was deemed too dangerous, it was decided that an exploratory laparotomy was called for. The upper transverse abdominal incision was selected because through this approach almost all possible abdominal sites of chromaffin tumor can be satisfactorily explored. In regard to the anesthesia, ether was chosen as the safest agent possible; cyclopropane would naturally be contraindicated in the presence of circulating sympathomimetic compounds.

Some of the elinical laboratory findings are of interest. This patient may be considered as analogous to a clinical experiment in which nor-epinephrine and epinephrine have been continuously infused for a period of at least two years and four months, with sudden removal of the hormones. The opportunity was thus presented of studying some of the effects of the chronic administration of presumably large amounts of these substances in man as well as the effects of their discontinuance. According to recent work such as that of Corcoran and Page (17), epinephrine may stimulate the production of adrenocorticotropic hormone by the anterior pituitary. However neither cortin nor 17-ketosteroid excretion was elevated in this patient before operation; these values were almost identical to those obtained after recovery[3] (Table 3). Seventeen days after the operation and fifteen days after the withdrawal of all sympathomimetic drugs, a fall in cortin and a rise in alpha 17-ketosteroid excretion were found. These observations are quite difficult to interpret. The fall in cortin level was seen at a time well beyond that seen in many postoperative cases (18) and was not accompanied by a drop in 17-ketosteroid excretion. One hesitates to draw any conclusions concerning adrenal cortical function under these circumstances. Thorn *et al.* (19) have reported a case of pheochromocytoma in which the urinary 17-ketosteroid excretion was normal.

Although the hyperglycemia seen in the presence of the tumor (but normoglycemia on another occasion) followed by a normal fasting blood sugar level after the operation is probably due to the sympathomimetic substances alone, it is tempting to suggest that the elevated glucose tolerance curve seen preoperatively is related to a stimulated adrenal cortex, especially in view of the occurrence of clinical diabetes in cases of pheochromocytoma (20).

A similar dilemma exists in regard to the interpretation of the high urinary gonadotropin levels. It might be argued that the preoperative

[3] The urinary excretion studies were performed in the Department of Pharmacology by methods to be published later. The cortin determination is a periodate method. The gonadotropin assay was performed on immature mice.

elevation could be related to sympathomimetic stimulation of the anterior pituitary (21), but as the level was found to be just as high nearly four months after the operation and as the later clinical picture suggests the presence of hypogonadism, it would seem that this may be merely an incidental finding (Table 3). The 17-ketosteroid levels, both alpha and beta, are not abnormal for a boy of his age. The present association of normal 17-ketosteroid and very high gonadotropin excretion in a 15-year-old hypogonadal boy is reminiscent of the cases of Heller and Nelson (22).

In view of the many theories of the etiology of hypertension, this case indicates that excessive administration of epinephrine and of nor-epinephrine can result in a chronic, sustained hypertension, which in itself apparently can produce retinal hemorrhages and choked discs; cerebral accident with increased cerebrospinal fluid pressure, headache, nausea, vomiting, convulsions, and coma; renal insufficiency with a low concentrating capacity, and urinary findings of red cells, white cells, and casts. Presumably the cardiac enlargement and electrocardiographic signs of myocardial damage were partly due to the direct, chronic action of the sympathomimetic substances on the heart.

The demonstration of the presence of nor-epinephrine and epinephrine in the tumor provides confirmatory evidence for previous reports. It was gratifying to find that the concentration of nor-epinephrine as found by biologic assay (0.86 mg.) and by paper chromatography (1.04 mg. per gram) checked so well. Much higher concentrations of nor-epinephrine in this tumor have been reported (4, 7, 8); it is possible that our values might have been higher if the tumor had been extracted or frozen immediately upon its removal. After the acid-alcohol extract was made, however, it was shown that no deterioration in activity occurred over a period of two months.

SUMMARY

A case of pheochromocytoma is presented.

The patient was a boy who at the age of 12 showed symptoms of sustained hypertension with cardiac enlargement, retinal hemorrhages, renal insufficiency, and hypertensive encephalopathy. The symptoms were gradually progressive over a two-year period until the patient sustained a cerebral accident at the age of 15 years. The diagnosis was made by means of the Benzodioxane test. On laparotomy, a tumor of the right adrenal medulla was found and successfully removed.

Urinary cortin, 17-ketosteroid and gonadotropin levels were determined before and after removal of the tumor. The first two were found to be normal but the gonadotropin excretion was elevated. The glucose tolerance curve was elevated before operation, returning to normal afterwards.

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The tumor was extracted with acid-alcohol and assayed for pressor activity in atropinized dogs before and after Dibenamine. By this method it was concluded that nor-epinephrine was present in the extract, equivalent to a concentration of 0.86 mg. per gram of tumor tissue. Paper chromatography indicated the presence of nor-epinephrine equivalent to 1.04 mg. per gram of tumor and a smaller amount of epinephrine.

Acknowledgment

We wish to thank Mr. Willard Starnes for the 17-ketosteroid determinations and for help in the extractions, Miss Jean Cowsert for the gonadotropin assays, and Mrs. Bobby E. Teague for the creatinine determinations. Merck and Co., Inc. supplied the Benzodioxane and Smith, Kline and French Laboratories provided the Dibenamine.

ADDENDUM

Since this manuscript was submitted, another paper on pheochromocytoma with findings similar to those herein reported has appeared (Calkins, E.; Dana, G. W.; Seed, J. C., and Howard, J. E.: On piperidylmethyl-benzodioxane (933-F), hypertension, and pheochromocytoma, *J. Clin. Endocrinol.* **10:** 1–11 (Jan.) 1950).

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THE DIRECT MEASUREMENT OF I^{131} UPTAKE IN THE THYROID GLAND; FURTHER OBSERVATIONS*

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ALTHOUGH the usefulness of radioactive iodine (I^{131}) in the study of thyroid gland function and in the treatment of hyperthyroidism is well established, many problems exist in the absence of a satisfactory method for accurate direct measurement of I^{131} in the thyroid gland in situ. Many previous studies (1–8) have utilized urinary I^{131} excretion. Although the urinary I^{131} content can be accurately measured, the urinary I^{131} excretion is, at best, an indirect indicator of thyroid gland function. In edematous and in uncooperative patients considerable error may be encountered. In other studies (9–16) direct measurements have been made by external counting over the thyroid gland with a single Geiger-Mueller tube. This method demands highly accurate positioning of the patient. Errors due to variation in gland size and location (geometry, absorption and scatter) are large and methods for their correction are not satisfactory (17, 18) for general clinical use. In addition, with the data gathered by the above methods, various attempts have been made to solve the problem of thyroid gland uptake of radioactive iodine by mathematical analyses (19–21). The need remains, however, for a direct, simple, quantitative method for measurement of I^{131} uptake by the thyroid gland.

It was shown previously by us (22, 23) that quantitative measurement of I^{131} uptake in the thyroid gland could be achieved by external counting with a device utilizing four to six platinum cathode Geiger-Mueller tubes connected in a parallel electrical circuit and arranged in a horizontal plane about the neck. General utilization by other clinics has been hindered by the unavailability of platinum cathode tubes of appropriate characteristics (listed subsequently). It was felt, however, that the fundamental principles involved in this method warranted application to Geiger-Mueller tubes with other types of cathodes. The following characteristics of Geiger-

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Mueller tubes were deemed necessary: i) universal availability, ii) comparable threshold voltages, iii) plateaus of one hundred or more volts, iv) equal sensitivity and suitability for the gamma rays emitted by I^{131} (24) and v) operating lives sufficiently long to permit the measurement of therapeutic as well as tracer quantities of I^{131}.

Studies have been carried out with bismuth cathode and several different copper cathode Geiger-Mueller tubes. One of the copper tubes[1] was found to be satisfactory. In a previous paper (8) we have shown the suitability of four of these tubes, arranged as described subsequently, for measurement

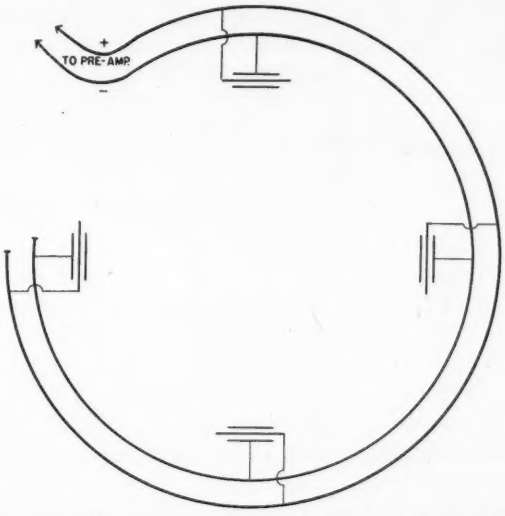

FIG. 1. Arrangement of four copper cathode Geiger-Mueller tubes in a parallel electrical circuit. A preamplifier (Tracerlab P-4) connected the four-tube circle to an autoscaler.

of urine excretion following therapeutic doses of I^{131}. It is our purpose to present its characteristics, comparing it to the platinum cathode Geiger-Mueller tube and demonstrating its suitability for quantitative measurement, by external counting, of thyroid gland I^{131} uptake following tracer and therapeutic doses in man.

MATERIAL AND METHODS

Characteristics of the copper cathode Geiger-Mueller tube

Four copper cathode Geiger-Mueller tubes (placed horizontally for ease of future shielding) were arranged in a circle and connected in a parallel

[1] GG 305 Sylvania Co. The cathode of this tube is a thin sheet of copper; that of the platinum tube is an 80-mesh screen.

electrical circuit (Fig. 1). One or more tubes could be excluded from the circuit. Coaxial cable (52-ohm) was used as the high voltage lead and radio hook-up copper wire was connected to the cathode and used as a ground. Voltage readings and counting rates were made on a Tracerlab autoscaler.

The data presented in Table 1 are representative of many individual tubes studied. Threshold voltages varied within a narrow range and plateaus were longer than 100 volts. Sensitivities were comparable (Table 1) as evidenced by background counts (tube shielded, *see* following section) varying between 61 and 65 per minute, and by the counting rate from a

TABLE 1. COMPARISON OF FOUR COPPER CATHODE GEIGER-MUELLER TUBES INDIVIDUALLY AND CONNECTED TOGETHER IN A PARALLEL ELECTRICAL CIRCUIT

Tube No.	Threshold voltage	Length of plateau (volts)	Background cts./min. (with shielding)	Net cts./min. 45 cm. from 440 μc source (with shielding)	Deviation from av. ct. rate %
1	1,235	>100	61	2,240	+3.5
2	1,260	>100	65	2,260	+4.5
3	1,240	>100	64	2,060	−4.5
4	1,235	>100	65	2,080	−3.5
4-tube circle	1,235	>100	250	8,860	+2.5

source of I^{131} deviating a maximum of ± 4.5 per cent from the average counting rate.

The characteristics of four of these tubes connected together (circle radius, 45 cm.) were similar (Table 1). The threshold voltage was 1,235 volts, the plateau was longer than 100 volts, the background counting rate was four times that obtained with a single tube and the net counts recorded from a source of I^{131} placed in the center of the circle were 2.5 per cent more than the sum of the net counts per minute measured by the four individual tubes (Table 1).

The four-tube circle

Four copper cathode Geiger-Mueller tubes were arranged in a circle (*see* preceding section) and experiments were carried out to determine the optimal circle radius. Two requirements were deemed essential, 1) adequate sensitivity and 2) an efficient "free circle." The latter has been defined (8) as the area concentric with the outer circle of Geiger-Mueller tubes within which a source of I^{131} may be moved with a small change (5 per cent) in the counting rate as compared to the counting rate obtained

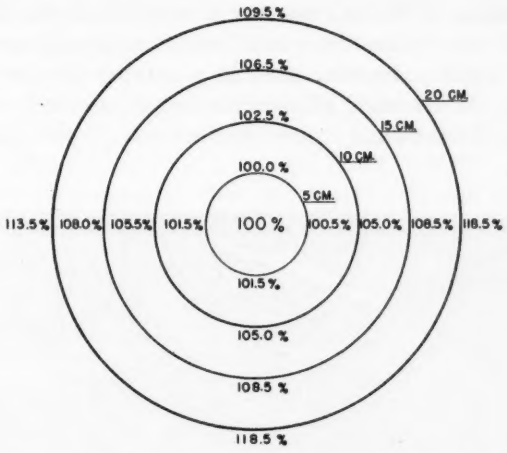

FIG. 2. Change in measured radiation from a source of I^{131} at various horizontal distances from the center of the shielded four-tube circle, radius 45 cm. *See* text for description.

with the source in the center of the circle. It has been previously shown (with platinum cathode tubes) that the radius of this inner circle bears a direct relationship (ratio = $1.0:3.5 \pm 0.5$) to the radius of the four-tube circle (8). In the present study, circles of three radii, 40, 45 and 50 cm., were examined (Table 2). Background counting rates were the same. The counting rate from a source of I^{131} was determined with the source in the center of the circle and at four horizontal poles around the center at distances of 5, 10, 15 and 20 cm. (Fig. 2). Net counts per minute with the source in the center of the circle were considered to be 100 per cent. The four-tube circle with a radius of 45 cm. was optimal (Table 2). Compared with a 50 cm. circle, the 45 cm. circle offered a 21 per cent increase in sensitivity, whereas the "free circle" was little altered (Table 2). Compared

TABLE 2. OBSERVATIONS ON FOUR-TUBE CIRCLES OF VARIOUS RADII

Shielding	Distance of tubes from center of circle (cm.)	Observed radiation from I^{131} source			"Free circle" (cm. radius)	Ratio = Radius of "tube circle" / Radius of "free circle"	Net counts/μc I^{131} (source in center of circle)	No. of μc I^{131} giving net counts/min. equal to background counts/min.
		Source in center	Source 15 cm. from center	Difference %				
		Net counts/min.						
none	50	1,960	2,130	9.0	11.5	4.3	14.0	31.0
none	45	2,440	2,720	11.5	12.0	3.8	17.0	25.0
none	40	2,970	3,510	18.0	9.0	4.4	21.0	20.5
2 inches	45	12,800	13,900	8.5	10.0	4.5	21.0	12.5

with a circle radius of 40 cm., the 45 cm. circle showed a 24 per cent decrease in sensitivity but its "free circle" was considerably larger.

Circles with eight tubes connected in a parallel electrical circuit were also examined. An increased efficiency was not afforded; relative to the four-tube circle, background counts and net counts per microcurie of I^{131} were doubled.

FIG. 3. The four-tube copper cathode circle. Inset shows one tube in detail.

The effect of shielding on sensitivity and on the "free circle"

Lead bricks, one inch thick, were arranged around each tube as a box (Fig. 3), an opening 10×26 cm. remaining. The shielding extended 11 cm. in front of the tube. With one and two inches of lead shielding there was a reduction in background counts of 33 per cent and 39 per cent respectively (Table 3). There was no further decrease when the opening was closed by lead, two inches thick.

The effect of shielding on the sensitivity and the "free circle" of the 45 cm. circle is shown in Figures 2 and 4 and Table 2. The background counting rate in these experiments was 260 counts per minute. Twenty-one net counts/min./microcurie were measured with a source of I^{131} placed in the

circle center (Table 2). Thus, 12.5 microcuries of I^{131} at the center of the circle gave a counting rate 100 per cent over background. With the tubes unshielded 25 microcuries at the circle center were required for a counting rate 100 per cent over background (Table 2). Shielding, therefore, resulted in a 100 per cent increase in sensitivity over the unshielded four-tube circle, 45 cm. radius (Table 2).

The radius of the horizontal "free circle" (Fig. 2) was 10 cm. With the source 15 cm. from the center of the circle the counting rate was 8.5 per cent higher than that measured with the I^{131} source in the circle center

TABLE 3. EFFECT ON THE BACKGROUND COUNTING RATE OF SHIELDING THE FOUR-TUBE CIRCLE

Shielding (inches of lead)	Window (cm.)	Background (counts/min.)	Per cent reduction in background
0	—	115	—
1	10×26	77	33
2	10×26	70	39
2	none	70	39

(Fig. 2). Similarly, with perpendicular movement of the source in the circle center, the radius of the "free circle" was also 10 cm. (Fig. 4); 15 cm. above or below the tube level, there was a 10 per cent decrease in counting rate as compared to the counting rate when the source was placed in the circle center (Fig. 4).

The net counting rate with increments of I^{131}

Amounts of I^{131} varying from 116 microcuries to 16.2 millicuries were diluted with water to volume in a 25 cc. volumetric flask. The counting rate was measured with the I^{131} source in the center of the shielded four-tube circle, 45 cm. radius. There was a linear relationship between net counts per minute and the amount of I^{131} up to a counting rate of approximately 60,000 counts per minute, as obtained from a 3.2 millicurie source (Fig. 5). With stronger sources of I^{131} the net counting rate fell below the expected net counting rate, which was obtained by extrapolation from the linear portion of the curve. The loss in measured radiation from a 7 millicurie source was 5 per cent and from a 14 millicurie source, 20 per cent (Fig. 5). Because of differences in resolving time in the copper cathode Geiger-Mueller (99305) tube (6), this experiment should be carried out with each set of tubes.

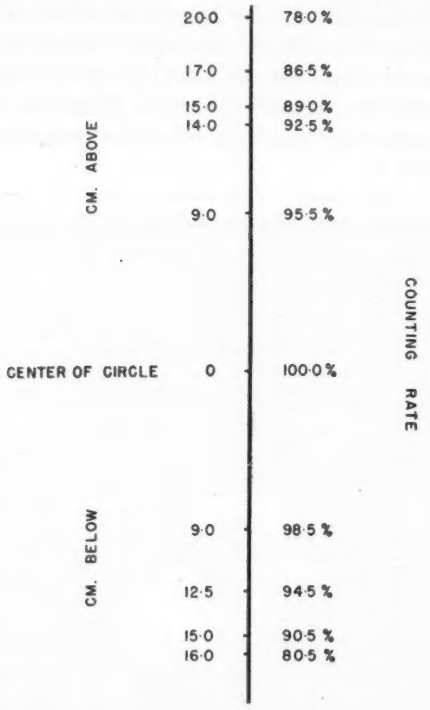

FIG. 4. Change in measured radiation from a source of I^{131} at various distances from center in the perpendicular axis of the four-tube circle.

Mock-up experiments

To assess the effects of absorption and scatter on the measurement of thyroid gland sources of I^{131}, various experiments were performed. First, to simulate cervical structures, water, meat and bone were placed about the I^{131} sources (70 microcuries to 2.46 millicuries) suspended in the center of the shielded four-tube circle.[2] The I^{131} solution was diluted with water to 10 and 50 cc. volumes. When the source was surrounded by meat, water and bone, the measured radiation (Table 4) differed from that measured from the unsurrounded source by an average of 1 per cent (range −3.0 per cent to +5.0 per cent). The differences observed were not related to the volume of the I^{131} source (Table 4).

In a second set of experiments, sealed rubber models were made to approximate the size and shape of 25 and 100 gram thyroid glands and to

[2] The shielded four-tube copper cathode circle, 45 cm. radius, hereafter will be referred to as C.C.C.; the shielded four-tube platinum cathode circle, 60 cm. radius, as P.C.C.

TABLE 4. SIMULATION BY MOCK-UP EXPERIMENTS OF THYROID GLAND SOURCES OF I^{131}. GROUP A REPRESENTS EXPERIMENTS CONDUCTED WITH THE I^{131} SOURCE DILUTED IN A TOTAL VOLUME OF 50 CC. OF WATER; GROUP B, IN 10 CC. OF WATER

Exp. No.	Tissue surrounding I^{131} source	I^{131} source					
		70 μc		183 μc		2.46 mc	
		Net counts per min.	Variation from A_1 %	Net counts per min.	Variation from A_1 %	Net counts per min.	Variation from A_1 %
A	1. None	1,410	—	3,580	—	47,000	—
	2. One-liter "water-jacket"	1,480	+5.0	3,580	0.0	45,500	−3.0
	3. One-inch "meat-jacket"	1,410	0.0	—	—	47,500	+1.0
	4. A_2 plus A_3	1,430	+1.5	3,580	—	48,000	+2.0
	5. A_4 plus "cervical vertebrae"	—	—	3,700	+3.5	—	—
B	1. None	—	—	3,590	0.0	47,500	+1.0
	2. One-liter "water-jacket"	—	—	3,650	+2.0	46,500	−1.0
	3. B_2 plus one-inch "meat-jacket"	—	—	—	—	48,000	+2.0
	4. B_3 plus "cervical vertebrae"	—	—	3,660	+2.0	—	—

contain 100 microcuries of I^{131} in total volumes of 15 and 50 cc., respectively. The radiation from each model was measured in the P.C.C. and the C.C.C. and considered to be 100 per cent. In 10 subjects the model was strapped with scotch-tape over the anterior surface of the neck in the position of the thyroid gland; the measured radiation was compared to that obtained from the source alone (Table 5). In the C.C.C. the counting rates were increased 7 per cent (average) by the presence of the subject; in the P.C.C., 5 per cent (S.E. of the difference between the means equals 0.85). No correlation could be drawn between the per cent differences encountered and the size of the model or neck size of the subjects.

In a third set of experiments a Levine tube containing 45 to 80 microcuries of I^{131} sealed between two lead beads (1.5 inches apart) was passed into the esophagus of 4 subjects. With fluoroscopic visualization the I^{131} source was placed opposite a marker previously placed on the skin over the thyroid gland (Fig. 6). The subject was positioned so that the source would be in the approximate center of the "circle." Counting rates from the tube source, both outside and within the subjects, were measured in the P.C.C. and the C.C.C. (Table 6). With the I^{131} source in the subject, the counting rate was increased by approximately 8.5 per cent in the P.C.C. and 6.5 per cent in the C.C.C. (Table 6).

The protocol of a fourth experiment in patient J. K. is shown in Table 7. The thyroid gland uptake measured by the C.C.C. twenty-four hours after the oral administration of 282 microcuries of I^{131} was 43.0 per cent; at the time of external measurement, 51 per cent of the dose had been excreted in the urine. Within two and one-half hours after the external measurement, a subtotal thyroidectomy was carried out and the radioactivity in the surgical specimen measured by the C.C.C. Thirty-one per cent of the administered dose was present in the specimen. Twenty-nine hours after surgery

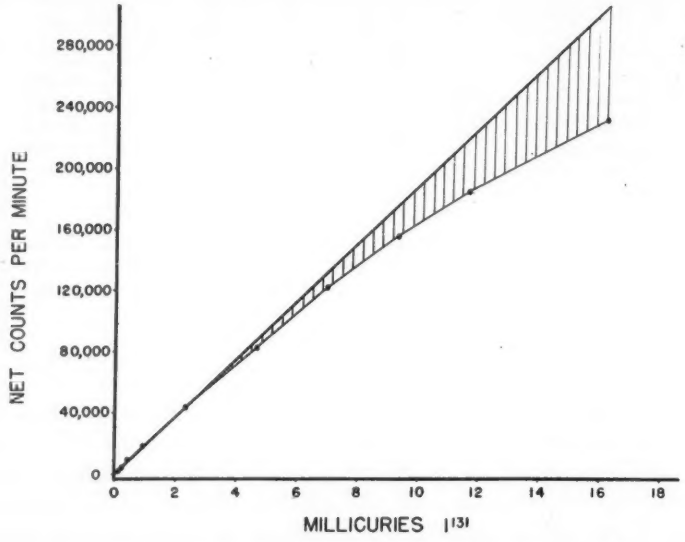

FIG. 5. The counting rate with increments of I^{131}. The straight line represents the expected counting rate.

the I^{131} in the thyroid gland region was remeasured by the C.C.C.; the thyroid radiation was 6 per cent of the administered dose. Measurement of washings from the surgical equipment, sponges, etc. accounted for an additional 1 per cent. Thus, of 2,210 net counts originally measured in the thyroid gland area, 1,980 or 90 per cent were accounted for.

RESULTS

Comparison of the thyroid gland uptake of I^{131} as measured in vivo by the C.C.C. and the P.C.C.

Ninety-six comparisons of thyroid gland uptake as measured by the C.C.C. and the P.C.C. were made in 25 consecutive patients (Table 8). The patients were classified into four groups: 1) hyperthyroid, 2) euthyroid,

TABLE 5. EFFECT OF ABSORPTION AND SCATTER ON THE MEASURED RADIATION FROM I^{131} SOURCES PLACED IN SEALED RUBBER MODELS OF THYROID GLANDS. THE MODEL WAS STRAPPED TO THE ANTERIOR SURFACE OF THE NECK IN THE POSITION OF THE THYROID GLAND. THE COUNTING RATE OBTAINED WITH THE MODEL SUSPENDED IN THE CIRCLE CENTER WAS CONSIDERED TO BE 100 PER CENT

Subject	Change in counting rate (%) when source is strapped to subject's neck			
	P.C.C.		C.C.C.	
	I^{131} source in 25 Gm. model	I^{131} source in 100 Gm. model	I^{131} source in 25 Gm. model	I^{131} source in 100 Gm. model
1	+10.0	+ 8.0	+ 9.0	+ 8.0
2	+ 7.5	+ 5.0	+ 5.0	+10.0
3	+ 4.0	+ 5.0	+ 4.5	+ 9.0
4	+ 3.0	+ 4.5	+ 6.5	+10.0
5	+ 3.0	+ 5.0	+ 5.0	+10.0
6	− 0.5	+ 1.0	+ 3.5	+ 5.5
7	+ 6.5	+ 9.0	+ 7.0	+ 8.0
8	+ 8.5	+10.0	+ 9.5	+ 8.5
9	+ 4.5		+ 6.0	
10	+ 8.0		+ 5.5	
11		− 1.5		+ 4.0
12		− 0.5		+ 4.0
Ave.	+ 5.5	+ 4.5	+ 6.0	+ 7.5
Combined average	+5.0 (S.D. 3.1)		+7.0 (S.D. 2.2)	

TABLE 6. EFFECT OF ABSORPTION AND SCATTER ON THE MEASURED RADIATION FROM I^{131} SOURCES IN A LEVINE TUBE, PASSED TO THE LEVEL OF THE THYROID GLAND IN 4 SUBJECTS

Subject	P.C.C.			C.C.C.		
	I^{131} source alone	I^{131} source in patient	Increase in counting rate %	I^{131} source alone	I^{131} source in patient	Increase in counting rate %
	Net counts per min.			Net counts per min.		
J. M.	3,290	3,590	8.5	1,235	1,290	4.5
W. M.	4,280	4,580	7.0	1,580	1,730	9.5
G. L.	2,580	2,900	11.0	990	1,100	11.0
I. L.	2,470	2,660	7.5	900	910	1.0
			Av. = 8.5			Av. = 6.5

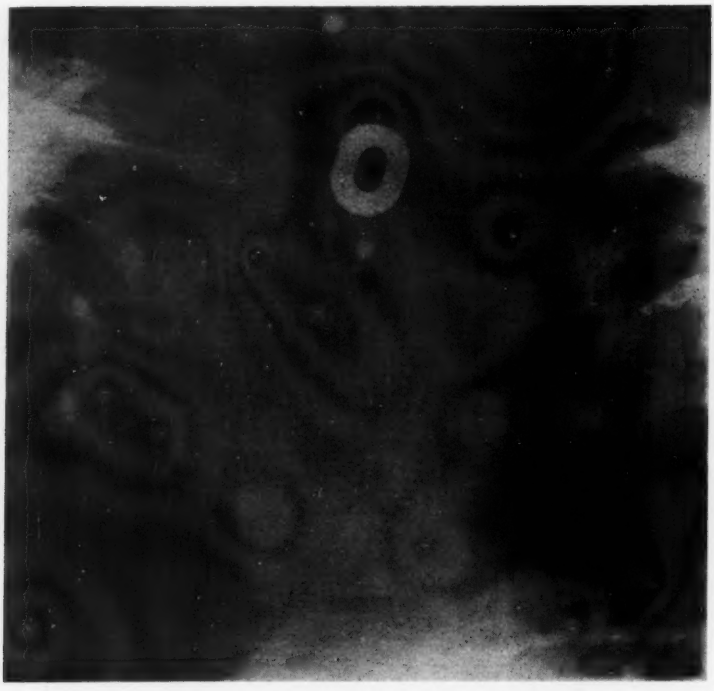

FIG. 6. A roentgenogram in patient W. M., showing the position of an I^{131} source in the esophagus at the level of the thyroid gland. *See* text for description.

TABLE 7. AN IN VIVO EXPERIMENT DEMONSTRATING THE ACCURACY OF THE MEASUREMENT BY THE C.C.C. OF THE I^{131} THYROID GLAND CONTENT

Date	Hour		Net counts/min. at time of measurement	Net counts/min. corrected to time of preoperative measurement	Preoperative neck counts/min. accounted for %	Tracer dose accounted for %
12/13/49	9:00 A.M.	282 μc I^{131} administered		(5,140)*		
12/14/49	9:00 A.M.	Patient placed in C.C.C.	2,210			(43.0)
	10:30 A.M.	Subtotal thyroidectomy begun				
	11:30 A.M.	Right lobe and isthmus of thyroid gland (34 grams)	1,520	1,535	69.5	30.0
		Piece of left lobe (1 gram)	55	55	2.5	1.0
		Total counts from operating equipment, sponges, etc.	50	50	2.5	1.0
12/15/49	2:00 P.M.	Patient placed in C.C.C.	270	340†	15.5	6.0
		1st 24-hour urinary excretion				51.0
		2nd 24-hour urinary excretion				5.0
		Totals		1,980	90.0	94.0

* Calculated from standard.

† Corrected also for turnover rate.

3) hypothyroid and 4) miscellaneous. Clinical estimation of thyroid gland function was based on history, physical examination and laboratory data including serum cholesterol and basal metabolic rate. The relationship of the uptake studies to thyroid function and previous I^{131} therapy (Cases 2, 3, 4, 6, 7, 11, 12, 22, 24) will be discussed in a subsequent paper (25). Neck circumference, length of neck and thyroid gland size and position were estimated in each patient. One hundred and fifteen to 150 microcuries of I^{131}, carrier-free, were administered orally to the fasting patients. The glass container was rinsed twice with 50 cc. of water; the washings were administered. (The measurement in the P.C.C. of the rinsed glass container showed insignificant radiation.) At varying intervals thereafter, such as twenty-four, forty-eight and seventy-two hours, the thyroid gland uptake was measured, first, in the P.C.C. and, immediately following, in the C.C.C. The patient was placed so that the anterior surface of the neck at the level of the thyroid isthmus was approximately 1 cm. in front of the circle center. Slight movements from this position incurred no significant error as evidenced by repeated measurements of the radiation. The time interval for 4,096 or 2,048 counts was measured; infrequently 1,024 counts were determined. Two or more measurements were made in each circle, the average of which was used in the calculations; the maximum deviation of repeated measurements from the average was less than 2 per cent. The per cent uptake by the thyroid gland was calculated by relating the radiation from the thyroid gland to the radiation from the standard, a 50.0 cc. aliquot of the I^{131} stock solution from which the patient's dose was taken. The following formula was employed:

$$\text{Thyroid gland uptake } \% = \frac{\text{net counts/min. thyroid gland}}{\dfrac{\text{cc. } I^{131} \text{ sol. administered}}{\text{cc. } I^{131} \text{ sol. in standard}} \times \text{net counts/min. standard}} \times 100$$

The thyroid gland uptake was converted from per cent to microcuries by multiplying the former by the number of administered microcuries, corrected for decay to the time of the external measurement. Urine, collected for 24-hour periods in bottles containing sodium hydroxide and toluene, was analyzed for I^{131} content by a method previously described (6).

The performance of the autoscaler, operated at 100 volts over threshold, was repeatedly checked by referring the counting rate of the standard measured frequently throughout the day to the predicted decay slope of I^{131}.

TABLE 8. COMPARISON OF THE THYROID GLAND UPTAKE AND BIOLOGIC HALF-LIFE FOLLOWING TRACER DOSES OF I^{131} AS MEASURED BY THE FOUR-TUBE, PLATINUM CATHODE AND COPPER CATHODE, CIRCLES

Case No., Initials	Additional diagnoses	Estimated thyroid gland weight (grams)	B.M.R. %	I^{131} tracer dose (microcuries)	Days after tracer	*Platinum cathode circle*			*Copper cathode circle*			Diff. in measurements by P.C.C. and C.C.C. of thyroid uptake %	Cumulative urinary excretion %	Tracer accounted for* %		Biologic half-life (days)		
						Thyroid gland uptake		Ratio: Neck	Thyroid gland uptake		Ratio: Neck							
						%	microcuries	background (cts./min.)	%	microcuries	background (cts./min.)			P.C.C.	C.C.C.	P.C.C.	C.C.C.	Difference %
GROUP I. HYPERTHYROID																		
1. G.F.	Chronic thyroiditis	65	+15	150	1	42.5	58.0	12.5	43.0	59.0	5.5	+ 1.0	49.5	92.0	92.5	4.6	4.4	− 4
					2	40.5	51.0	11.3	41.5	52.5	4.9	+ 2.5	52.0	92.5	93.5			
					3	36.5	42.0	9.8	36.5	42.0	4.2	0.0						
					6	28.0	25.0	6.2	28.5	25.5	3.0	+ 2.0						
2. A.R.	None	40	+12	150	1	69.0	94.5	19.4	65.5	89.5	7.8	− 5.0	31.0	100.0	96.5	5.8	5.8	0
					2	65.0	82.0	17.2	67.5	85.0	7.4	+ 4.0	32.5	97.5	100.0			
					3	63.5	73.5	16.2	60.0	69.5	6.2	− 5.5						
					12	40.0	21.0	5.4	39.5	21.0	2.6	− 1.0						
3. L.M.	None	45	+22	150	1	49.0	67.0	14.3	49.5	68.0	6.2	+ 1.0	31.5	80.5	81.0	2.6	2.9	+12
					2	35.5	44.5	9.8	37.5	47.0	4.7	+ 5.5	37.0	72.5	74.5			
					3	31.5	36.5	8.5	33.0	38.0	3.7	+ 5.0	41.0	72.5	74.0			
					6	22.5	20.0	5.0	23.0	20.5	2.6	+ 2.0						
4. C.L.	Rheumatic heart disease	55	+15	150	1	73.5	100.5	20.9	72.0	98.5	8.5	− 2.0	16.5	90.0	88.5	3.6	3.8	+ 6
					2	61.5	77.5	16.2	66.0	83.0	7.6	+ 7.5	19.0	80.5	85.0			
					3	55.0	63.5	14.2	55.5	64.0	5.0	+ 1.0						
					5	49.0	47.5	10.3	49.0	47.5	4.6	0.0						
					8	39.0	29.5	7.1	38.5	29.0	3.2	− 1.5						
5. E.C.	None	35	+27	150	1	68.5	94.0	19.6	65.0	89.0	7.8	− 5.0	34.5	103.0	99.5	4.8	5.3	+10
					2	65.0	82.0	17.1	66.5	84.0	7.3	+ 2.5	37.5	102.5	104.0			
					3	60.0	69.5	15.5	57.5	66.5	6.0	− 4.0						
					5	55.0	53.5	10.6	54.0	52.5	4.9	− 2.0						
					8	45.5	34.0	8.1	48.0	36.0	3.7	+ 5.5						
6. H.W.	Incomplete remission following I^{131}	Not palpable	+ 4	150	1	49.5	68.0	14.3	48.0	66.0	6.0	− 3.0	48.0	97.0	96.0	4.3	4.9	+14
					2	46.0	58.0	12.4	48.0	60.5	5.6	+ 4.5	50.5	96.5	98.5			
					3	42.5	49.0	11.3	42.5	49.0	4.7	0.0						
					5	35.0	34.0	7.6	36.5	35.5	3.7	+ 4.5						
7. A.M.	None	25	+19	150	1	55.5	76.0	16.1	55.5	76.0	6.8	0.0	39.5	95.0	95.0	4.0	4.2	+ 5
					2	48.5	61.0	13.0	49.0	62.0	5.9	+ 1.0	42.0	90.5	91.0			
					3	45.0	52.0	12.0	46.0	53.0	5.0	+ 2.0						
					5	39.0	38.0	8.3	39.0	38.0	3.9	0.0						
					9	28.0	19.0	5.1	31.0	21.5	2.6	+10.5						
8. S.C.	None	55	+14	137	1	49.5	62.0	13.2	48.0	60.0	5.6	+ 3.0	48.0	97.5	96.0	6.4	6.2	− 3
					2	46.5	53.5	12.2	44.5	51.0	5.1	− 4.5						
					4	45.5	44.0	10.0	42.0	40.5	4.1	− 7.5						
					8	41.0	28.0	6.7	40.5	27.5	3.1	− 1.0						

9. R.B.	None	40	+40	145	1 2 3 7 8	89.0 83.0 78.5 69.5 66.5	118.5 101.5 88.0 55.0 48.0	26.0 22.6 19.2 12.5 10.5	87.5 82.0 75.5 69.0 66.5	116.5 100.0 84.5 54.5 48.0	10.3 9.0 7.5 5.3 4.8	− 1.5 − 1.0 − 4.0 − 0.5 0.0	16.0 17.5 18.0	105.0 100.5 96.5	103.5 99.5 93.5	5.0	5.0	0
10. S.J.	Nodular goiter	40	+40	115	1 2 5 6	75.5 74.5 67.5 65.5	79.5 72.0 50.0 45.0	17.7 16.5 11.6 10.0	76.0 73.0 62.5 63.5	80.0 70.5 46.5 43.5	7.2 6.5 4.9 4.4	+ 0.5 − 2.0 − 7.5 − 3.0	24.5 26.0 27.0	100.0 100.5 94.5	100.5 99.0 89.5	6.2	5.6	−10
11. M.L.	None	65	+40	150	1 2 4 6	64.5 60.5 57.0 55.0	88.5 76.0 60.5 49.0	20.0 17.3 13.8 11.8	61.0 59.5 54.0 53.5	83.5 75.0 57.0 47.5	7.6 6.9 5.6 5.1	− 5.5 − 1.5 − 5.5 − 2.5	16.5 19.0 19.5	81.0 79.5 76.5	77.5 78.5 73.5	5.6	5.6	0
12. R.S.	None	>100	+24	126	1 2 5	97.5 93.0 79.0	112.5 98.5 65.0	24.8 22.2 13.9	91.5 93.0 79.0	105.5 98.5 65.0	9.4 8.1 6.1	− 6.0 0.0 0.0	6.5 8.0	104.0 101.0	98.0 101.0	5.8	5.4	− 7
GROUP II. EUTHYROID																		
13. J.B.	Paroxysmal auricular fibrillation	25	+ 12	145	1 2 3	30.5 32.0 31.5	40.5 39.0 35.5	9.4 9.3 8.2	32.0 32.0 34.0	42.5 39.0 38.0	4.4 4.1 3.9	+ 5.0 0.0 + 8.0	49.5 56.5 57.5	80.0 88.5 89.0	81.5 88.5 91.5	8.4	8.0	− 5
14. S.S.	Angina pectoris	25	+ 1	145	1 2 3 7	32.5 36.0 34.0 32.5	43.0 44.0 38.0 25.5	10.2 10.3 8.9 6.5	32.0 33.5 34.0 32.5	42.5 41.0 38.0 25.5	4.4 4.3 3.9 3.0	− 1.5 − 7.0 0.0 0.0				8.4	8.4	0
15. A.H.	Meningo-vascular syphilis	25	—	135	1 2 3 6	25.5 26.5 26.5 25.0	31.5 30.0 27.5 20.0	7.6 7.1 6.9 5.1	24.5 25.5 26.0 24.5	30.0 29.0 27.0 19.5	3.4 3.2 3.0 2.5	− 4.0 − 4.0 − 2.0 − 2.0	54.5 60.0 61.0	80.0 86.5 87.5	79.0 85.5 87.0	8.4	8.0	− 5
16. E.F.	None	25	− 1	135	1 2 6 8	23.0 23.0 21.5 18.5	28.5 26.0 17.5 12.5	7.0 6.3 4.5 3.4	25.0 21.5 21.0 20.5	31.0 24.5 17.0 14.0	3.4 2.9 2.3 2.0	+ 8.5 − 6.5 − 2.5 +11.0	45.0 55.0 57.5	68.0 78.0 79.0	70.0 76.5 78.5	6.4	6.4	0
17. F.S.	Peripheral vascular insufficiency	25	—	135	1 2 3	14.0† 13.0 12.0	17.5 15.0 12.5	4.5 4.1 3.6	15.5 12.0 12.5	19.0 13.5 13.0	2.5 2.1 2.0	+11.0 − 8.0 + 4.0	65.0	79.0	80.5	—‡	—	—
18. R.F.	Hypertrophic arthritis	25	—	135	1 2 3	31.0 30.0 28.0	38.5 34.0 29.0	8.9 8.1 7.0	31.5 30.0 29.0	39.0 34.0 30.0	4.2 3.6 3.4	+ 1.5 0.0 + 3.5	52.5	83.5	84.0	—	—	—

TABLE 8.—*Continued*

Case No. Initials	Additional diagnoses	Estimated thyroid gland weight (grams)	B.M.R. %	I^{131} tracer dose (microcuries)	Days after tracer	*Platinum cathode circle*			*Copper cathode circle*			Diff. in measurements by P.C.C. and C.C.C., of thyroid uptake %	Cumulative urinary excretion %	Tracer accounted for* %		Biologic half-life (days)		
						Thyroid gland uptake		Ratio: Neck	Thyroid gland uptake		Ratio: Neck							
						%	microcuries	background (cts./min.)	%	microcuries	background (cts./min.)			P.C.C.	C.C.C.	P.C.C.	C.C.C.	Difference %
19. J.B.	Iodine-deficient goiter	75	−11	135	1	41.0	50.5	11.7	39.0	48.0	4.8	− 5.0				7.6	6.9	− 9
					2	41.0	46.5	10.5	38.5	43.5	4.3	− 6.0						
					3	41.5	43.0	10.2	39.5	41.0	4.1	− 5.0						
					6	38.5	31.0	7.4	37.0	30.0	3.2	− 4.0						
20. R.S.	Iodine-deficient goiter	65	+ 3	135	1	65.5	81.0	18.1	65.5	81.0	7.2	0.0	36.0	101.5	101.5	8.0	8.8	+10
					2	64.0	72.5	15.8	64.0	72.5	6.5	0.0	37.0	101.0	101.0			
					3	64.5	67.0	14.2	66.5	69.0	6.3	+3.0						
					7	63.0	46.5	11.0	63.0	46.5	3.6	0.0						
21. R.A.	Nodular goiter	70	− 1	150	1	52.5	72.0	13.2‖	53.5	73.5	6.6	+ 2.0	43.5	96.0	97.0	7.2	7.5	+ 4
					2	55.5	70.0	14.8	55.5	70.0	6.2	0.0	46.0	101.5	101.5			
					3	51.0	59.0	13.3	52.0	60.0	5.5	+ 2.0						
					5	52.5	51.0	11.0	50.5	49.0	4.7	− 4.0						
22. V.E.	None	25	−11	147	1	37.5	50.5	11.1	37.0	50.0	5.0	− 1.5	42.5	80.0	79.5	5.5	5.5	0
					2	36.0	44.5	10.4	33.5	41.5	4.2	− 7.0	44.5	80.5	78.0			
					5	32.5	31.0	7.5	31.0	29.5	3.5	− 4.5						
GROUP III. MYXEDEMA																		
23. E.S.	Carcinoma of the thyroid gland	Not palpable	−12	150	1	14.0	19.0	4.7	14.0	19.0	2.5	0.0	68.5	82.5	82.5	3.5	3.8	+ 9
					2	12.0	15.0	4.0	12.0	15.0	2.2	0.0	77.0	89.0	89.0			
					3	11.0	12.5	3.6	10.5	12.0	1.9	− 4.5	78.0	89.0	88.5			
					5	11.0	10.5	2.7	10.5	10.0	1.8	− 4.5						
GROUP IV. MISCELLANEOUS																		
24. I.L.	Incomplete remission from I^{131}‖	Not palpable	+10	137	1	8.5	10.5	3.1	9.5	12.0	1.9	+12.0	65.5	74.0	75.0	—	—	—
					2	7.0	8.0	2.6	8.0	9.0	1.8	+14.5	78.5	85.5	86.5			
					3	7.5	8.0	2.6	7.0	7.5	1.6	− 6.5						
25. N.S.	Malignant exophthalmos¶	Not palpable	− 5	135	1	5.5	7.0	2.4	5.5	7.0	1.5	0.0	76.0	81.5	81.5	—	—	—
					3	3.0	3.0	1.6	3.0	3.0	1.3	0.0						

* Cumulative urinary excretion plus thyroid gland uptake.
† Iodine medication given by dentist two weeks prior to tracer.
‡ Insufficient points.
§ Background high at time of measurement.
‖ KI less than three weeks prior to tracer.
¶ Desiccated thyroid, 120 mg. daily.

The results of the comparison of the measurement of thyroid gland uptake by the P.C.C. and the C.C.C. are presented in Table 8 and Figures 7 and 8. The thyroid gland uptake at the end of twenty-four hours ranged from 5.5 per cent to 97.5 per cent as measured by the P.C.C., and 5.5 per cent to 91.5 per cent as measured by the C.C.C. (Table 8). The average difference of the 96 comparisons was −0.25 per cent (range −7.5 per cent to +14.5 per cent, S.D. 3.1). There was no relationship between the per cent difference in uptake measured by the two methods and the estimated

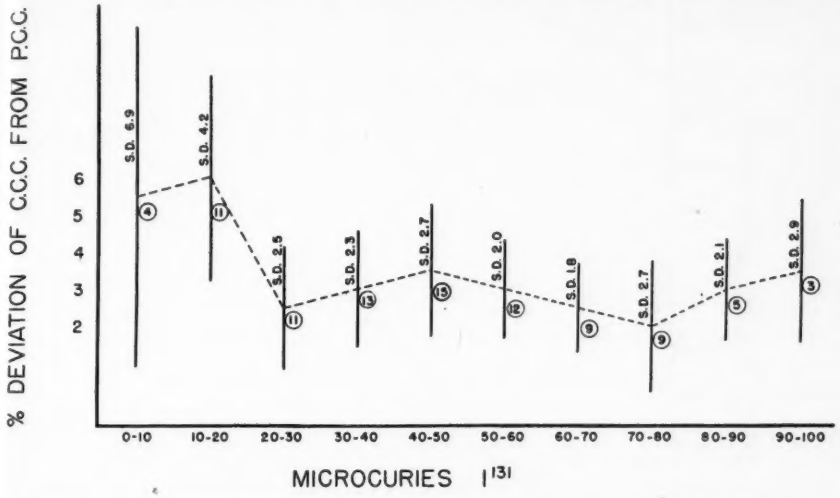

FIG. 7. The per cent deviation of the C.C.C. measurement from the P.C.C. measurement of the thyroid gland uptake of various increments of I^{131}. The average deviations are connected by the broken line. The vertical bar represents the standard deviation. The encircled numbers represent the number of determinations.

weight (varying between normal and over 100 grams) and position of the thyroid gland or the volume and configuration of the neck (Table 8).

The relationship between the per cent difference of the two measurements and the I^{131} thyroid gland content is demonstrated in Figure 7. With I^{131} quantities greater than 20 microcuries the average difference was under 3.5 per cent; with I^{131} quantities below 20 microcuries, 6 per cent.

Difference in sensitivity of the C.C.C. and P.C.C. In patients with hyperthyroidism the ratio of neck counts/min. to background counts/min. measured by the C.C.C. twenty-four hours after administration of the tracer dose was above 5.5; for the P.C.C. this ratio was above 12.5 (Table 8). In the 25 patients, the ratio ranged from 1.5 to 10.3 in the C.C.C., and 2.4 to 26.0 in the P.C.C. (Table 8). Similarly, Figure 8, relating net thyroid gland counts as measured by the P.C.C. and the C.C.C. to the amount of

I^{131} present in the thyroid gland, demonstrates the difference in sensitivity of the two circles, the P.C.C. being 2.5 times more sensitive than the C.C.C.

Comparison of biologic half-life as measured by C.C.C. and P.C.C. The biologic or effective half-life (26, 27), defined as the number of days for the amount of I^{131} present in the thyroid gland to decrease by 50 per cent, was calculated when four or more readings had been obtained over a period of five or more days (Fig. 9). The biologic half-lives varied from 2.6 to 8.4 days as calculated from measurements by the P.C.C. and 2.9 to 8.8

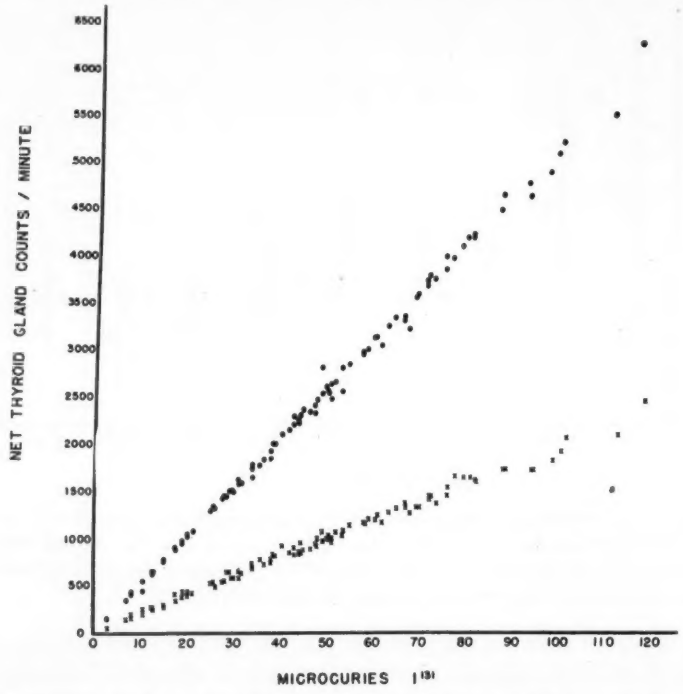

FIG. 8. Comparison of the sensitivity of the C.C.C. and the P.C.C.—the results of 96 determinations of thyroid gland uptake in 25 patients.

days as measured by the C.C.C. (Table 8). The average difference for 21 comparisons of biologic half-life determinations was 5.5 per cent (S.D. 4.3).

Measurement by the C.C.C. of the thyroid gland uptake after tracer and therapeutic doses of I^{131}.

The applicability of the C.C.C. to studies following the administration of therapeutic doses of I^{131} is shown in Table 9. In 10 patients, twenty-four hours after the administration of tracer doses, the thyroid gland uptake

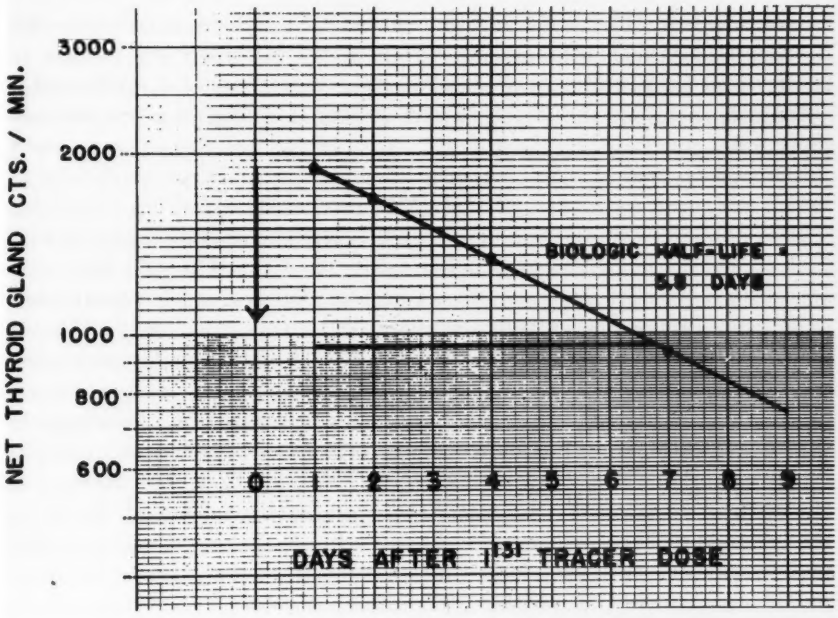

FIG. 9. A biologic half-life (Patient H. S.) calculated from counting rates measured in the C.C.C.

TABLE 9. COMPARISON OF THE THYROID GLAND UPTAKE AS MEASURED BY THE C.C.C. AFTER TRACER AND THERAPEUTIC DOSES OF I^{131}

Patient	Thyroid gland uptake of I^{131} in 24 hours		
	Tracer dose %	Therapeutic dose %	Difference %
R.B.	89	88	1
S.C.	48	43	10
S.J.	76	66	13
M.L.	61	54	11
L.M.	50	54	8
A.R.	65	70	8
C.L.	72	72	0
C.O.	87	91	5
A.M.	79	73	8
B.H.	91	82	10
		av. = 7.4 (S.D. 4.2)	

varied between 48 per cent and 91 per cent. After therapeutic doses (4.0 to 13.5 millicuries) administered to the same patients, the uptake in twenty-four hours varied between 43 per cent and 91 per cent. The average difference of the uptake of tracer and therapeutic doses was 7.4 per cent (S.D. 4.2).

COMMENT

It is apparent from the data presented that the results obtained with four copper cathode Geiger-Mueller tubes (Sylvania Co. GG 305) connected in a parallel electrical circuit and arranged in a horizontal circle of 45 cm. radius are entirely comparable to those obtained with the platinum cathode tube circle. These have been in use in our laboratory for eight months and sixteen months respectively and their relative advantages and disadvantages may be briefly summarized. The advantages of the copper cathode tube circle are i) uniformity and ready availability of the tubes, ii) usefulness for measurements of radiation after tracer and therapeutic doses of I^{131}, iii) satisfactory sensitivity for tracer studies in euthyroid and thyrotoxic individuals and iv) applicability to measurement of I^{131} excretion in urine, feces and sputum following therapeutic doses (8). So far as we have been able to determine, the only disadvantage is the lower sensitivity of this tube as compared to the platinum cathode Geiger-Mueller tube. Compared under identical circumstances, the platinum cathode Geiger-Mueller tube is five times as sensitive as the copper cathode tube (28). However, under the conditions described in this paper,the P.C.C. is two and one-half times as sensitive as the C.C.C. This makes possible the measurement of very low thyroid gland uptakes with a higher accuracy in the P.C.C. than is feasible with the C.C.C. The larger tube circle employed with the platinum cathode tube has not been shown by these studies to be of paramount importance. It is apparent from the results that a 45 cm. radius circle is sufficiently large to measure accurately the radiation from thyroid glands varying in size from normal to at least five times normal (Table 8). A considerable increase in sensitivity of the P.C.C. would be observed by using a 45 cm. circle. At the present time the disadvantages of the platinum cathode tube are lack of uniformity and unavailability. We have, therefore, limited the use of the P.C.C. to the measurement of I^{131} quantities up to 150 microcuries. The application of more sensitive Geiger-Mueller tubes or scintillation counters to the four-tube method may further increase the latter's sensitivity and make possible the use of a considerably smaller tracer dose.

It would appear from a review of the literature (16, 17) and our personal experience that the hitherto utilized methods of measurement of thyroid gland uptake are subject to an error of at least ± 15–20 per cent. The

present studies show a markedly increased accuracy of the measurement of the thyroid gland uptake of I^{131} by the four-tube method. The main source of error is attributed to the effects of absorption and scatter. The observed radiation from an I^{131} source in proximity to a subject's thyroid gland when measured by the C.C.C. was too high by 7 per cent; when measured by the P.C.C., 5 per cent. The influences of various nonthyroid sources of I^{131} on its measurement in the thyroid gland are considerably less. From data on circulating I^{131} (29–31) it was estimated that at the end of twenty-four hours, in both euthyroid and thyrotoxic patients, the total circulating I^{131} was less than 1.5 per cent of the tracer dose. Assuming the thyroid blood flow to be 25 per cent (probably high) of the total cardiac output, less than a 0.5 per cent increase in the measured radiation from the thyroid gland due to circulating I^{131} in euthyroid and thyrotoxic individuals would be expected. The error due to I^{131} in the urinary bladder was investigated. Radiation from sources placed at the position of the urinary bladder was 2 per cent of that from the same source placed in the center of the circle. Likewise, when a source of I^{131} (75 μc) equal to that calculated to be in the patient's thyroid gland (such an amount of I^{131} would not be expected to be in the urinary bladder at any one time) was held at the patient's symphysis pubis, the net counting rate was increased by 5 per cent. The counting rate from indifferent areas equal in volume to the neck (usually the low thigh region) was also determined. In a thyrotoxic patient six hours following a tracer dose, the radiation from the thigh was 0.5 per cent of that from the neck. In patients with complete myxedema, in whom a higher circulating I^{131} might be anticipated at twenty-four hours, we have observed uptakes of 6 per cent in twenty-four hours following a tracer dose, whereas after seventy-two hours there was virtually no detectable radiation from the neck. It has been tentatively concluded from the foregoing considerations that the measurement of thyroid gland I^{131} content by the four-tube method is too high by approximately 8 per cent. Considering the error in this estimate to be ± 3 per cent (Tables 5 and 7) and the possible error in operation to be no more than ± 2 per cent, we have tentatively concluded that the four-tube method offers accuracy to ± 5 per cent.

SUMMARY AND CONCLUSIONS

A new method for the quantitative measurement of thyroid gland uptake of I^{131} is described. The instrument consists of four copper cathode Geiger-Mueller tubes connected in a parallel electrical circuit and arranged horizontally in a circle, radius 45 cm. Each tube is shielded in a two-inch-thick lead box, a window 10×26 cm. remaining. The "free circle," previously described as a circle concentric to the outer tube circle, within

which a source of I^{131} can be moved with a change in counting rate of less than 5 per cent, has a radius of 10 cm. The radiation from 12.5 microcuries of I^{131} was equal to that recorded as background; this sensitivity allows the use of 100 to 150 microcuries as a tracer dose. Mock-up experiments have demonstrated that the determination of thyroid gland uptake by external counting with the four-tube circle, after tracer and therapeutic doses of I^{131}, is in excess by approximately 8 per cent. The measurement, thus corrected, is accurate to ± 5 per cent.

Studies of thyroid gland uptakes after tracer doses of I^{131} as measured by the platinum (shielded four-tube, platinum cathode, circle radius 60 cm.) and copper circles showed an average difference of -0.25 per cent (S.D. 3.1) in 96 comparisons in 25 patients. No correlation could be drawn between this per cent difference and the estimated weight and position of the thyroid gland or the volume and configuration of the neck. Biologic half-lives were determined from measurements in both "circles"; in 21 comparisons these differed by 5.5 per cent (S.D. 4.3).

The thyroid gland uptake as measured by the C.C.C., following tracer and therapeutic doses of I^{131} was studied in 10 patients. The 24-hour uptake following therapeutic doses of I^{131} differed by an average of 7.4 per cent (S.D. 4.2) from the per cent uptake twenty-four hours following tracer doses.

The method here described offers quantitative accuracy, reduplicability and simplicity in studies of thyroid gland uptake and turnover following tracer and therapeutic doses of I^{131}.

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PREGNANCY TEST WITH THE MALE FROG (*RANA RIDIBUNDA*)

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LITERATURE

IN 1947, Mainini (1) described a simple and rapid test for the diagnosis of pregnancy in women, employing as criterion spermatogenesis in the male toad (*Bufo arenarum* Hensel). At the same time, Robbins, Parker and Bianco (2) described the same reaction in the male South African clawed frog (*Xenopus laevis*). In 1948 Mainini's results were confirmed by Haines (3). According to Bregman (1949, (4)) the use of Palestinian toads and frogs for pregnancy tests seemed to be feasible.

In view of these findings, we were encouraged to study the possible utilization of the Palestinian toad, *Bufo viridis*, for pregnancy diagnosis with human urine (1949 (5)). Our results, which were published in 1949 (6) showed that because of the low sensitivity of the toad to gonadotropic hormone, about 10 per cent of cases with a positive reaction may be wrongly diagnosed as negative, especially when the gonadotropin content is low. We then decided to study the use of Palestinian *Rana ridibunda* for this purpose, and our results with this frog are reported in the present paper.

MATERIALS AND METHODS

Our experiments were performed on male frogs (*Rana ridibunda*) varying in weight from 20 to 50 grams. Many of them were recently caught and others were kept in captivity for two to three months. After such a period, only a few of the frogs survived, in spite of having been maintained in a suitable environment. It is noteworthy that, contrary to our expectations, toads survived only one month on an average.

Our experiments with *Rana* were performed during the months of March through December, 1949, since toads were difficult to collect during this season.

The test animals can be used repeatedly, and it has been our custom to rest the animals for seven days after the completion of a test with a positive reaction. As a matter of fact, spermatozoa appeared within one half to two hours and disappeared within twenty-four to forty-eight hours after the inoculation with pregnancy urine. [In the toad they appeared within one to

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three hours.] A rest period of three to five days was considered sufficient after a test with negative results. It should be stressed that the sensitivity of the frogs decreases after repeated use, and results which were possibly falsely negative had therefore to be checked by the injection of a urine which had given a positive reaction in another frog. In addition the test was repeated in another frog.

The animals were kept in a cool spot separately in glass jars containing tap water 3 cm. deep. The water was renewed daily in each jar. Male frogs were easily recognized by the wart on the dorsum of the thumb. This is black when the animals are first caught, but the pigment sometimes vanishes during captivity.

Since it is impossible to determine the exact age of frogs collected in the field, we tested the sexual maturity of every frog brought into the laboratory. A frog reacting to 10 I.U. of chorionic gonadotropin with extrusion of sperm was considered fit for further use. Although frogs weighing less than 20 grams may give a sperm reaction, we did not use them.

Injections were given with a 15-gauge record needle, which was introduced through the skin of one of the hind legs into the muscular layer, and from there was pushed forward until it reached the dorsal lymph sac. This method avoided leaking of the urine through the skin wound. The animals received doses of untreated filtered urine ranging between 1 and 4 cc. One cc. was given as a routine dose. If it proved to be insufficient, as attested by a parallel AZ test with a positive result, the dose was increased. Practically, every frog withstood the injection of 2 cc. but 4 cc. could be injected only into the larger ones.

Sperm was removed from the cloaca with a dropper pipette, usually at the time of the injection and one hour thereafter. If the result was negative, sperm was again collected three and twelve hours after the injection. Sperm, free of contamination by stool and worms, is easily obtained by the following technique: The rubber bulb of the pipette should not be very large. It is bent at a right angle to the pipette when introduced, and is released when the pipette is within the cloaca. The slight negative pressure obtained by this technique is quite sufficient to suck the urine into the pipette. Release of urine is provoked by pressing the end of the pipette firmly against the dorsal fornix of the cloaca at its most caudal point, so as to bend the caudal end of the spine outward. This technique does not, however, work in the toad.

As a rule, it was found that the result arrived at after one to two hours is the most reliable, and is not improved by further readings. Results were classified by rough quantitative estimation of the number of spermatozoa per microscopic field (×200) as follows:

1 to 5: ±
6 to 10: +
11 to 20: ++
21 to 50: +++
51 to 100: ++++

Every result from + to ++++ was interpreted as a positive reaction.

Evaluation of the results followed the lines laid down in our earlier papers (Zondek, Sulman and Black, 1945 and 1948, (7, 8); Sulman and Sulman, 1950 (6)). Every case was controlled by the rapid hyperemia modification of the AZ test as well as by the original AZ test carried out on infantile female rats. Furthermore, every case was followed up clinically. Every student of pregnancy tests knows that the reliability of a test method can not be assessed by studying 100 patients with positive reactions and 100 patients with negative reactions. Critical testing for reliability must include a large number of very early pregnancies, disturbed pregnancies, tumors and cysts in the material analysed. In the latter cases gonadotropin excretion is substantially reduced. We have classified our case material according to the differential diagnosis furnished by the clinician. Furthermore, we have excluded women attending our social welfare clinics in the second or third months of normal pregnancy, during which period gonadotropin excretion in the urine reaches a natural peak. In this latter type of case all pregnancy tests show a high grade of reliability due to the large amount of chorionic gonadotropin in the urine.

RESULTS

We have studied 500 cases as classified in Table 1. The results can be summarized as follows: The 500 cases studied consisted of 163 subjects with a negative response, 143 normal pregnancies, 22 extrauterine pregnancies, 81 threatened abortions, 41 missed abortions, 31 moles and chorionepitheliomata including follow-up examinations, 17 miscellaneous genital tumors, 1 pituitary tumor and 1 corpus luteum cyst verified by the AZ test and followed clinically. In only 423 of these cases could the diagnosis be made by the male frog test. This represents an accuracy of only about 85 per cent.

Among the 163 cases with a negative reaction were 5 (3 per cent) with a false positive result. These 5 reactions were only slightly positive, designated with one + according to our table; but even so, they were misleading. Robbins and Parker (9) found that epinephrine may elicit sperm reactions in frogs, similar to those evoked by gonadotropic hormone. Hence, it may be that in our 5 cases a similar compound was excreted with the urine.

TABLE 1. CASE MATERIAL

Diagnosis	Total of cases examined		Number of wrong responses to frog test	Percentage of wrong responses to frog test
Cases with negative reactions (including abortions with negative response to the AZ test)	163		5*	3.0*
Normal pregnancies	143			
a) menses delayed: 5–10 days		30	22	73.3
b) menses delayed: 10 days–4 months		105	0	0
c) menses delayed: more than 4 months		8	3	37.5
Extrauterine pregnancies	22			
a) ruptured		16	12	75
b) undisturbed		6	0	0
Threatened abortions	81			
a) miscarriages		28	10**	35.7**
b) brought to term		53	15	28.3
Missed abortions	41			
a) first examination		33	8	24.2
b) second examination		8	0	0
Moles and chorionepitheliomata (including follow-up examinations)	31		0	0
Miscellaneous genital tumors	17		0	0
Pituitary tumor with positive response to AZ test	1		1	100
Corpus luteum cyst with positive response to AZ test	1		1	100
Total	500		77	15.4

* The wrong frog-test results all showed slightly positive reactions, designated: +.
** The wrong frog-test results actually predicted fetal death.

Chorionic gonadotropin, if found in amounts of less than 333 I.U. (which is in our rats, 1000 R.U.) per liter of urine, does not elicit a positive response to the pregnancy test in the male frog, but it does so in the infantile female rat. Follicle-stimulating hormone (prolan A), in the concentrations occurring in untreated urine of women in the menopause, in very early pregnancy, or after abortion yields no frog reaction.

In cases with increased gonadotropin excretion (more than 100,000 R.U. or 33,333 I.U. per liter of urine in cases of twins, mole and chorionepithelioma) frogs reacted to 0.1 cc. of urine instead of to 1.0 cc. Our material was, however, not extensive enough to permit definite conclusions as to the diagnostic reliability of these quantitative estimations.

A titration of chorionic gonadotropin in male frogs and in infantile female rats showed that the amount of chorionic gonadotropin detectable

with rats in the hyperemia test, or by the vaginal smear and luteinization tests is one fifth of that detectable by the male frog test or by the toad test.

DISCUSSION

The lack of sensitivity of the male frog to chorionic gonadotropin is a simple explanation for the high percentage (15 per cent) of false negative results. Table 1 clearly shows that the male frog test is fully reliable with all clear-cut cases of pregnancy and in most subjects showing a negative reaction. A hormone laboratory, however, consulted for reasons other than sheer curiosity, will obtain very disappointing results with the insensitive frog test. All cases with reduced excretion of gonadotropin will pass unnoticed, such as for instance early pregnancies (73 per cent false negatives) late pregnancies (37 per cent false negatives), ruptured extrauterine pregnancies (75 per cent false negatives), threatened abortions brought to term (28 per cent false negatives). The wrong results obtained in such cases with the frog test represent a distinct failure. In other cases, however, the "false" negative reaction with the frog test actually predicted the real outcome, thus representing an advantage rather than a failure, *e.g.*, with the threatened abortions terminated by miscarriages (35 per cent) and missed abortions (24 per cent). Even in these cases, fetal death could also be predicted with the quantitative rat-ovary hyperemia test in spite of its positive reaction, because the use of titration in this test offers the advantages of a graded response, permitting prognosis (8). Moreover, the rat test, which also permits the determination of follicle-stimulating hormone (prolan A) informs the clinician whether the supposedly aborting patient has actually been pregnant, a conclusion which cannot be derived from a negative reaction to the frog test.

Since the frog is only one fifth as sensitive as the rat to chorionic gonadotropin, an attempt might be made to overcome this difficulty by injecting larger quantities of urine into the frog; but this procedure is limited by the fact that more than 2 cc. of urine may kill the frog. Concentration of the urine by alcohol precipitation is the only alternative.

SUMMARY

1. The reliability of the male frog pregnancy test, using the Palestinian *Rana ridibunda* as the test animal, was studied in 500 cases.

2. In only 85 per cent of the cases with a positive reaction could the diagnosis be made with the aid of this test. In comparison, the accuracy of the toad test is 90 per cent; and that of the Aschheim-Zondek test is 99 per cent.

3. The 15 per cent of failures described with the frog test consisted chiefly of false negatives. Only 3 per cent of false positives were encoun-

tered, all of which were no more than slightly positive and therefore suspicious. This result is due on the one hand to the rather specific nature of this test, and on the other to the large amount of chorionic gonadotropin needed to elicit a positive reaction in the male frog.

4. Five infantile female rat units of chorionic gonadotropin are equivalent to 1 adult male frog unit and to 1 toad unit.

5. Hence, the pregnancy test with the male frog can be used only as a supplement to other methods of pregnancy diagnosis. It is valuable because of its rapid response within one half to two hours, whereas the Aschheim-Zondek test with the rat generally takes one half to four days. The frog test alone cannot, however, be relied upon, especially in disturbed pregnancies, because of its high percentage of false negative results (15 per cent) and because of occasional false positive results (3 per cent).

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STUDIES ON ADRENAL FUNCTION IN SPRUE: PRELIMINARY REPORT

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EVER since Verzar (1) suggested the possibility of adrenocortical hypofunction as an etiologic factor in celiac disease of children and adults, many investigators have speculated about a similar possibility for sprue. Investigation of this theory has not been undertaken until now in our institution because all efforts have been concentrated on the therapeutic angle. The only pertinent information we could find was a personal statement by Koppisch who, in a review of 1,400 consecutive autopsies, found 17 cases of sprue. The duration of the disease could not be ascertained in 5 cases. In the others it varied from four months to five years. Histologic sections were available from 13 of these cases, alterations being found in all but one, which was normal. The periadrenal fat showed serous atrophy, generally advanced. The cortical lipoids had undergone depletion and the zona glomerulosa and fasciculata were atrophic. This condition was most marked in the superficial portions of the cortex, where the cords of cells were usually very narrow. The interstitial tissue of the atrophied parts was broadened by edema. In the case with the most advanced atrophy there was actual loss of portions of the cords of cells, which had been replaced by very loose and edematous areolar tissue. These alterations were not specific, since they resembled those found in other cases of cachexia.

The technical complications inherent to endocrine research, acted as a further deterrent, but fortunately the simple test for adrenal insufficiency devised by Thorn and his co-workers (2) and later modified by Recant *et al.* (3) on the basis of Long's experiments (4), offered an opportunity to undertake a preliminary study.

MATERIAL AND METHODS

Ten patients were selected from several institutions and carefully studied. They met all the diagnostic criteria for sprue, including the bone-marrow picture. Eight of them had never received specific treatment

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in the form of liver extract, folic acid or vitamin B_{12}, and the other 2 were in state of relapse after several months without treatment. The test was performed before any medication was given.

It was decided to employ Thorn's modified technique using epinephrine, because the study had already been held up for over a year due to our inability to obtain ACTH. Furthermore, our own experience with the single-injection method (5) convinced us that it was a satisfactory screening test. The procedure was further simplified by limiting it to a determination of eosinophils and lymphocytes, and a total white cell count.

Initial total and differential white cell counts and an absolute eosinophil count were made in every case, followed by the subcutaneous administration of 0.3 ml. of a 1:1,000 epinephrine hydrochloride solution. Total and differential counts were repeated two hours after injection and absolute eosinophil count four hours after injection. Lymphocyte counts were calculated from the total and differential counts. The patients remained fasting throughout the entire test period.

RESULTS AND DISCUSSION

The results are summarized in Table 1. In 9 out of 10 cases of proved, untreated sprue a satisfactory response was obtained as evidenced by a decrease in circulatory eosinophils, which varied between 40 and 92 per cent. The only case in which there was only a 21 per cent decrease was one complicated by the presence of schistosomiasis and syphilis. The former probably accounted for the high initial eosinophil count and possibly the high-normal total white cell count. In the other 2 cases in which the initial eosinophil count was over 360, intestinal parasitosis was not ruled out.

TABLE 1

No.	Patient	Sex	Age (yrs.)	Eosinophil count			Lymphocyte count			Total WBC	
				Before	4 hrs. after	% decrease	Before	2 hrs. after	% change	Before	2 hrs. after
1	R.M.*	M	50	256	143	44	1,172	540	+53.9	3,350	4,700
2	P.A.	F	26	448	206	54	4,252	3,146	−26	11,150	11,200
3	P.V.	F	28	173	93	47	2,602	954	−63.4	8,100	10,150
4	A.R.	M	40	168	100	41	2,145	1,875	−20	3,350	2,600
5	R.C.	M	36	50	12.5	75	1,725	2,714	+50	2,300	3,050
6	E.D.	F	26	50	12.5	75	1,384	1,069	−23	3,550	3,450
7	N.F.	M	18	312	118	66	1,665	3,264	+96	4,500	5,100
8	A.M.**	M	22	1,787	1,381	21	5,365	4,158	−27	10,950	9,450
9	P.E.	M	38	81	6.25	92	2,250	2,135	− 5	7,500	7,350
10	L.R.	F	33	444	276	40	2,257	1,174	−48	6,100	4,350

* Relapse; no treatment for several months.

** Schistosomiasis and syphilis.

It is noteworthy that, with the exception of Cases 8, 9 and 10, all other sprue patients had initial total white cell counts of less than 5,000.

The extremely variable response of the lymphocytes may be explained by the fact that their release into the blood stream is more rapid and variable than that of the eosinophils. Thorn (6) stresses these findings in a recent publication and states that the lymphopenia is transitory even in the presence of continued steroid output.

On the whole, our results in this very limited series seem to indicate a normal capacity of the adrenal cortex to respond to stimulation by endogenous ACTH in uncomplicated untreated sprue.

SUMMARY

1. A preliminary study of adrenocortical function in untreated sprue is reported.

2. The method used was the determination of adrenocortical response to stimulation by endogenous ACTH as described by Thorn *et al.*

3. The results seem to indicate a satisfactory response in untreated, uncomplicated sprue.

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Endocrine Review

THE ROLE OF THE FETAL ENDOCRINE GLANDS IN DEVELOPMENT*

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Outline

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I. INTRODUCTION

THE many problems suggested by the title represent an aspect of endocrinology that is primarily characterized by neglect, and to the writer's knowledge has been brought forth for discussion only incidentally. The problems are of fundamental importance in relation to the physiology of development, to general endocrinology, to the biology of sex, and to basic problems of cancer.[1] Whereas any discussion at the present time will lead to no degree of finality, it appears worthwhile to focus attention on some

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[1] Topic for discussion at the 1949 Gordon Research Conference on Cancer.

of these problems if only to point out some of the inadequacies in our present knowledge. Until they are well defined and some of the evidence is assembled to suggest a part of the available information, the accumulated observations will continue to be scattered, uncoordinated, uncritically evaluated and lacking in direct focus upon the central problem. The purpose of this discussion, therefore, will be to point out the types of approach that have been made and to assemble a part of the available data and some of the speculations, but it will be in no sense an attempt to cover the litterature exhaustively. Certain areas of the problem have received greater attention than others, and this is especially true for the sex hormones and their possible role in sexual differentiation. The interested reader is referred to such recent reviews as those of Price (131), Jost (90), Moore (114), Courrier (39), Burns (24) and Ponse (123).

Some basic considerations may be suggested as fundamental in an approach to the study of the effects of hormones during development in vertebrates.

1. There should be a determination of the time when a given endocrine gland could be supposed capable of synthesizing specific hormones from the materials provided through the blood stream. Histology, cytology and cytochemistry of progressively developing endocrine organs have been relied upon in great measure to suggest the functional state of the structure, but in many cases interpretations of observations have remained equivocal. The responses of the organ in question to experimental procedures of an indirect nature have been of considerable aid but again are often inconclusive.

2. A more dependable criterion than structural organization to denote the capacity of the gland to elaborate its hormone is the actual detection of effective substances through transplantation of living tissue, or the injection of extracts of embryonic tissues into a suitable host. This may involve hosts of the same species, frequently of different age than the donor, or it may involve intact hosts, or such separate tissues as isolated heart, uterine muscle strip or others denoting response, from species other than the donor.

3. The determination of capacity to secrete, and indeed the presence of detectable hormone in the gland, are still insufficient to assure any degree of endocrine activity in the intact, developing embryo, since to be effective in the organism as a whole the active substance must be released from the organ which produces it. One may recall that a larval amphibian may carry a thyroid gland which, if macerated in situ, will induce metamorphosis in the animal by virtue of the hormone released and thus made available to it, whereas when undisturbed it would not effect metamorphosis until a considerably later time. Or, again, an individual immature female mouse

or rat may fail to show any obvious effects from a gonadotropic substance from its own pituitary, but when the latter organ is macerated and implanted subcutaneously into a littermate sister, or even a younger female, it may precipitate marked gonadotropic activity in the host. Releasing mechanisms must be operative in order that a hormone secreted and stored in the gland of an embryo may be available for any possible effect in the intact animal. The release may be into a circulating system whereby the secretory product is widely distributed and, when in sufficient concentration, can affect target organs at some distance. Less widely appreciated is the fact that in some cases release may be by diffusion through tissues thus accounting for more nearly local effects. The "cortexin" and "medullarin" reactions of Witschi (183) represent a case in point, although these "activator" substances demonstrated in the gonads of amphibia are believed to be different from the usual steroidal sex hormones secreted by the gonads. Jost (89) has demonstrated that embryonic rat testis transplants placed on seminal vesicles of mature castrated hosts secrete steroidal hormones and thus exert local androgenic stimulation through diffusion. The writer, in unpublished experiments, has confirmed this claim.

4. Finally to be considered is the capability of end organs or tissues to respond to such hormonal substances as may reach them. The reproductive tract of the immature female mouse or rat may show phenomenal response to freshly implanted pituitary substance around the age of 20 days, whereas a few days earlier it would show but little if any response (Smith and Engle (153)). The threshold of response on the part of a given target organ is an important matter, as is also the gradual increasing sensitivity of the organ to respond to the hormone. This increasing sensitivity of the target organs of the reproductive system has been demonstrated in postnatal rats by Price and Ortiz (132) and in postnatal hamsters by Ortiz (119) (*see* discussions of Price (131)). During prenatal life the embryo in some cases may show marked responses to sex hormones when these are injected into the pregnant mother (*rat*—Greene, Burrill, and Ivy (59–62); *mouse*—Raynaud (136, 137), Turner (160, 161); *guinea pig*—Dantchakoff (42); *monkey*—Van Wagenen and Hamilton (162)) whereas in other cases practically no response occurs in the embryo after entirely similar treatments (Bruner and Witschi (16); White (170, 171); Davis and Potter (46)). It should be further pointed out that the sensitivity of, and the character of response by a given tissue or organ to hormones that reach it, undergo change; a tissue may show definite response when one grade of development has been attained only to lose it, or respond in a different manner, at a later stage in development (113).

An influence of hormones during development, therefore, cannot be expected to occur until a) the endocrine organs, themselves in process of

differentiation and development, have attained a developmental state that permits them actively to manufacture their hormones; b) physiologic conditions within the organism have reached a state of development that permits release of hormone from the gland, and provides for hormone distribution sufficient to bring the target organs under its influence; and c) the target organs themselves have attained a degree of differentiation that permits their effective response to the activating substance.

A further complicating and confusing situation in an analysis of the developmental influences arising within an embryo relates to the possible influence from hormones from extraneous sources; the fetus of placental mammals is bathed in hormonal substances present in amniotic fluid, and after parturition further exposures to maternal hormones may be provided through nursing. These problems have been ably discussed by Price (130, 131) in relation to sex differentiation. That unsuspected influences may enter through milk has been recently emphasized from the demonstration that cancer in certain strains of mice is dependent upon the transmission of some factor ("milk-factor") through the milk from an affected mother to her offspring (9, 10).

A final point in generalities and emphasis may be made before proceeding to a more detailed examination of evidences for hormonal activity during development; this involves the general tendency to refer definite responses to the period of birth, or to a fetal and a postnatal period. Such reference must be considered appropriate only as dating a period for descriptive purposes since, from the developmental aspects, it is meaningless as a basis of comparison between species. The stage of development at birth among mammals ranges from a tiny, helpless animal still in an embryonic condition among marsupials, to that of a well developed individual capable of running about, as is found among guinea pigs, cattle, sheep, horses and others; on the basis of differentiation of particular organs or systems there is the widest variation. Birth is thus only an incident in the developmental life of an animal, not an index of a definite attainment in differentiation.

II. PITUITARY HORMONES

A. Histologic evidence of pituitary secretion

In the anterior lobe of the pituitary gland the acidophils and basophils are presumed to be the sources of the various hormones elaborated by this lobe. Accordingly, considerable attention has been given to studies on embryos to determine at what period these cells become differentiated.

In the chick, Rahn (133) has determined that both acidophil and basophil cells occur on the tenth day of incubation; however, fully differentiated basophils are said not to occur until after hatching and are especially

associated with the occurrence of the onset of sexual maturity. Among mammals there is general agreement that cellular differentiation of anterior lobe cells into acidophils and basophils occurs during early developmental stages—prenatal in placental mammals, and in early pouch-young stages in marsupials. The sequence in differentiation is not always the same, since acidophils appear earlier in some species, whereas in others basophils may be first to differentiate. This development of a chromophilic state is a gradual process rather than a precipitous one.

Australian marsupials of several species according to Parker (120), and the American opossum according to Wheeler (167), exhibit chromophilic differentiation about the eleventh to the eighteenth day of pouch-young life, while they are still in an early developmental stage (opossum snout-rump length approximately 30 mm.). The pig fetus develops cellular granulations in the anterior lobe cells from the 50 to 170 mm. fetal stage (116, 144), the horse fetus at a crown-rump length of 64 to 138 mm. (69), the cat at 75 mm. length (14), man during the third or fourth month (36) and the guinea pig about mid fetal life (30 da.) (94).

Thus presumptive evidence derived from the histologic appearance of developing glands would suggest the probability of the embyronic pituitary secretion of hormones at an early stage of development. Since, however, it is more convincing when actual physiologic data are available to denote the presence of active products in the gland, we may turn attention to some of the evidence available from such studies.

B. *Evidence from physiologic activity of tissues*

Smith and Dortzbach (152) sought for growth-stimulating substances in the pituitary of fetal pigs by implanting these glands into hypophysectomized rats. Positive growth occurred when the pituitary glands were removed from fetal pigs above 90 mm. in crown-rump length; no response was given when glands from 70 to 90 mm. length were employed but increasing amounts of growth-promoting substance were evident in larger fetuses. Only negative results were obtained on implantation of muscle or brain tissues from fetuses whose pituitaries were actively growth-stimulating.

Thyrotropic hormone from pituitaries of embryos has also been demonstrated. In the chick, Martindale (106) transplanted thyroid glands from chick embryos previously hypophysectomized in order to exclude any effect from the pituitary to the donor animal; one thyroid was transplanted onto the chorio-allantoic membrane of a normal host whereas the second hypophysectomized-chick-thyroid was placed on the membranes of an hypophysectomized host. The further development of the transplant from the hypophysectomized donor on the two hosts would thus demonstrate any

effect upon the graft carried by the normal host. By this approach it became evident that the pituitary in the normal host exerted a thyroid-stimulating effect upon the transplant as early as day 11. In pituitaries of the pig fetus, Rumph and Smith (144) found a thyroid-stimulating principle in the glands from embryos of 260 to 280 mm. crown-rump length. Extracts of these fetal pituitaries injected into hypophysectomized tadpoles stimulated their thyroid glands sufficiently to bring on metamorphosis, which would not occur without the presence of a thyroid-stimulating agent from the pituitary gland.

The presence of gonadotropins in embryonic pituitary glands was demonstrated by Smith and Dortzbach (152). Fetal pig pituitaries were implanted twice daily into immature mice of 18 to 21 days with autopsy on the fourth day of implantation. Gonadotropic activity was evident when pituitaries from fetuses of 200 to 210 mm. C–R length were employed; pronounced ovarian growth, vaginal membrane opening and uterine distension occurred in the recipient mice. Some evidence was obtained from pituitaries of somewhat younger fetuses but those under 170 mm. gave negative results for the gonadal-stimulating principle. In the dove pituitary, Riddle and Schooley (138) failed to obtain evidence for the gonadotropic principle in hatched but immature birds.

The melanophore-expanding principle of the pituitary, although specifically associated with the pars intermedia of the pituitary, takes origin from the oral lobe anlage and remains associated with the anterior lobe in those animals that fail to develop an intermediate lobe (chicken, armadillo, whale, etc.). The presence of this active principle (intermedin, melanophorotropin) has been demonstrated during embryonic development by transplantation and by injections of extracts. Hogben and Crew (71) injected extracts of the pituitary gland from the ox and sheep fetus into frogs and hypophysectomized axolotls and determined the presence of the active principle as early as the 3-month fetal stage; Snyder (154) detected it in the pituitary gland of the pig fetus of 30 mm. In the chick embryo, potent melanophore-expanding substances were obtained from extracts of the pituitary region of 5-day and older stages when the extracts were administered to hypophysectomized frogs (33); similar findings were recorded by Rahn and Drager (134) through injection of extracts into hypophysectomized chameleons. This test, developed by Kleinholz and Rahn (96) enabled Kleinholz (95) to detect the melanophoric principle from heads of 7 mm. free-swimming larvae of *Rana pipiens* when extracts of the bodies did not yield it; indeed, the effective melanophoric substance could be detected from extracts of tail bud stages of amphibian larvae. In *Rana esculenta* its presence was detected in embryos of 3–4 mm. (34).

Thus, through these and many other experiments, the presence of the

active principles issuing from the pituitary has been demonstrated during stages of embryonic development. It is of importance also to emphasize that the presence of the active physiologic principles has been frequently demonstrated prior to the differentiation of the gland. This has been pointed out for the pig by Snyder (154) Rumph and Smith (144), and in the frog by Kleinholz (95), Etkin (50) and by others.

It must be recalled that the presence of an active principle in the substance of a gland does not assure that these substances are being discharged into the organism or that they exert any effect upon development. More direct evidence for or against the conception that pituitary substances actually play a role during development has been provided through the removal of the pituitary by various methods. Such an operation on aquatic vertebrates is in many cases simple, but in the bird and mammal more difficulties are encountered and it is sometimes questionable whether one can assume that a rigorous operation involving the removal of considerable portions of an embryo has no other effect than the removal of the individual gland under consideration. It is understandable that not all authors arrive at the same conclusions relative to the embryonic effectiveness of pituitary secretions, particularly when different species are involved.

The amphibia in many ways have been animals of choice for such experimentation, due to the ease with which the oral lobe of the pituitary can be removed in the anlage stage; it is furthermore especially useful by virtue of the responsiveness of melanophores, and because of the phenomenon of metamorphosis. Gudernatsch (66) contributed an important technic by demonstrating that very tiny frogs could be produced from tadpoles in a brief interval by the feeding of fresh thyroid gland, and subsequent investigations have pointed out clearly the value of the metamorphic technic. The fundamental work of Smith (149 to 151) and Allen (1) on amphibian larvae, revealing pigmentary changes following removal of the anlage of pituitary oral lobe, has likewise provided important foundation stones for the study of embryonic endocrine function during development.

From the classic paper of Smith (151) it is clearly revealed that, in the amphibian embryo, ablation of the pituitary anlage demonstrates pituitary function during larval development. Pigmentary changes, absence of metamorphosis, growth disturbances, and the histologic state of thyroids and adrenals point to an effect during development of the melanophorotropic, growth, thyrotropic and adrenotropic hormones. Many additional investigations confirm and extend these findings.

In the chick embryo, destruction of the anterior portions of the head at an early stage of development by means of X-ray irradiation, with incuba-

tion continued until near hatching, was carried out by Wolff and Stoll (186) and by Stoll (156), while Fugo (55) surgically removed the head of embryos around the 12-somite stage and effectively prevented the appearance of the pituitary; such embryos developed in some cases up to the hatching age but could not escape from the shell.

The effects reported from these studies varied to some extent. Wolff and Stoll failed to note any obvious effects from the absence of the pituitary up to the stage of hatching or slightly earlier, thus inferring that the pituitary secretions were without effect during development. Treated animals and controls were of similar development and size and normal conditions prevailed for thyroids, thymus, adrenals, pancreas, liver, gonads, genital ducts, plumage and many other organs within the animal deprived of a pituitary. Fugo, on the other hand, while noting essentially normal development during early stages, believed that definite deficiencies were produced during the last half, thus denoting an absence of pituitary secretion. The average weight of hypophysectomized animals on the twelfth day was approximately 50 per cent of the controls; the testes showed some deficiency in interstitial cell development and in character of tubules; the left ovarian cortex was less developed than in controls, and late thyroid development showed interferences. There was no evidence of effects on genital duct development, or on thymus, adrenals, mesonephros, or parathyroids. Thus Fugo registers evidence that the growth factor, and the thyrotropic and gonadotropic factors were exerting effects during normal development and the deficiencies recorded indicated a lack of proper function in the chick embryo of these pituitary substances.

In mammalian development, Jost (90) in a review article mentions that decapitation of the rabbit fetus in utero exerts no effect upon sexual differentiation, but in a later article (91) he pointed out that whereas decapitation on day 22 caused no appreciable deficiencies, effects were noted when decapitation was done on days 18 or 19; these were attributed to a deficiency in gonadotropins, since administration of gonadotropins along with decapitation prevented such deficiencies as were noted in the reproductive system. In the fetal rat, Wells (165) presented suggestive evidence for removal of an adrenocorticotropin through the means of decapitation; he determined that such procedures led to an inhibition of growth of the adrenal with some modification in cortical cells, whereas similar decapitation accompanied by injections of an adrenocorticotropin preparation prevented the appearance of such adrenal changes.

Jailer (85), however, could find no evidence that the pituitary gland in newborn rats could respond to "stress" influences by the liberation of ACTH until about the eighth to the sixteenth day of age. Whereas the adrenal did not show a fall in ascorbic acid after injection of epinephrine

until the eighth postnatal day, nor was there a reduction in ascorbic acid as a result of exposure for two and one-half hours to a temperature of 5° C. until the sixteenth day, injections of ACTH on the fourth day did produce a fall of 34 per cent in ascorbic acid. It would appear that the pituitary is incapable of responding to stress influences by secretion of ACTH until several days after birth.

In hamsters, Foote and Foote (54) report decapitation of fetuses on the twelfth day of pregnancy and a study of the thyroid gland on the sixteenth day. Whereas the control revealed an increase in length of the thyroid gland of 79.3 per cent and an average increase in volume of 202 per cent during the intervening four-day period, the fetuses whose pituitary glands had been removed showed an increased length of thyroids of but 27.6 per cent and a reduction in volume of 17.8 per cent; few follicles and interstitial cells rather than differences in individual follicles accounted for the differences. Thus there was evidence for a function of thyrotropins at this stage of development.

Such experimental manipulations as have been performed on developing embryos, although not always yielding comparable results, do in the aggregate suggest that some influence may issue from the pituitary during development that plays a role in normal tissue differentiation and morphogenesis. Amphibia especially have given unmistakable results pointing to lack of certain of the active principles of the pituitary. In birds and mammals more radical procedures are required to gain removal of the developing gland and indicators for effects are not always too apparent, but some evidence does exist that the pituitary factors for growth-stimulation, and gonad-, thyroid-, and adrenal-stimulation play a role in normal differentiation.

It should be recalled that in the studies of Wright on the guinea pig certain inbred lines produced numerous offspring with abnormalities of the head, as well as headless individuals, that would assure in some cases an absence of the pituitary gland (189, 190); the specimens were obtained at birth but detailed presentation of internal anatomy was not given. It is to be pointed out, however, that on superficial examination no internal abnormalities of the body were found and that, except for defective heads, the animal was ordinarily plump and apparently normal. As a group the various grades of otocephalic monsters were 11 per cent lighter in weight than normal littermates—which the authors believe can be accounted for by the loss of the head weight alone. Among the group, there were reported 55 males and 26 females; hence the sex could readily be determined and no mention was made of sexual abnormalities. Since the guinea pig at birth is so mature, it is to be regretted that more data from these headless individuals are not available with respect to the internal anatomy, and especially on the condition of the endocrine organs.

Thus, although some evidence is available to suggest an influence of the pituitary gland secretions of the embryo upon differentiation and development, many more data are to be desired before attempting definitely to evaluate the role of these secretions upon specific developmental processes or to be very definitely assured of the stage in ontogeny at which the separate principles begin to play their separate roles in the organism.

III. THYROID

The problem of attempting an assessment of the role of thyroid secretions during embryonic development in higher vertebrates is made difficult because of the lack of demonstration of definite areas or structures upon which characteristic effects are exerted; the metabolic-stimulating effects, and influences on growth in later ontogeny, would be somewhat difficult to determine during developmental stages. Even in adult fish it is sometimes difficult to assign a very definite functional role for the thyroid gland. In goldfish, for example, Etkin, Root and Mofshin (51) claim that when mammalian thyroid substance makes up 50 per cent of the food intake, there is no influence upon the weight of these animals nor is there demonstrable metabolism-accelerating effects as would be indicated by increased oxygen consumption. In trout, however, Robertson (139, 140) has shown that secretions from the thyroid are responsible for skin color changes during the transition from the dark parr to the silvery smolt stage.

Amphibian development provides a dramatic demonstration of thyroid function in the transformation of the larva into the adult type. This phenomenon of metamorphosis, dependent upon thyroid function, can be used both as an example of the effects of thyroid hormone during this developmental period in the amphibian tadpole, and also as a means for detecting the presence of the hormone in glands of embryos from other species through either the implantation of the fresh gland substance or the utilization of extracts of the gland for injection.

The work of Gudernatsch (66) and Allen (2) revealed the multitude of complex changes that occur during metamorphosis, and the correlation of these detailed changes with the development of thyroid activity was pointed out by Etkin (49) and other investigators. In the frog larva, hind legs may develop and their growth keep pace for a time with growth of the body in general. At the period of accelerated growth of hind legs the thyroid demonstrates activity characterized by its own growth and by secretory activity which is largely governed by pituitary influences (2, 151). The definite onset of thyroid hormone function and effective release into the organism initiates the beginning of metamorphosis and regulates the sequence of events such as pronounced growth of fore and hind limbs, desiccation of tissues, tail resorption, changes in pigment pattern, the

changes in mouth parts, gut reduction and the other characteristic features (48). Such apparent effects among the amphibia, serving to indicate the beginning of functional activity of the thyroid, however, do not appear in the embryos of birds and mammals, hence other criteria for development of thyroid secretion must be utilized.

The chick thyroid gland starts its development at approximately the 48-hour stage of incubation and gradual development carries it to the first appearance of follicle formation and colloid droplet appearance in the cells; this has been shown to occur on the tenth or eleventh day of incubation (13, 32, 55, 72, 106, 157) and storage of iodine was detectable on day 10 (157). The further demonstration by Hopkins (72) that thyroids from chick embryos of 10 days' incubation would hasten metamorphosis of the amphibian larva proved the early formation of thyroid secretions in this gland. Hyperthyroidism can be induced after day 12 by injection of thyrotropin (188).

In fetuses of mammals, many observations indicate an early storage of iodine in the thyroid and the elaboration of a thyroid hormone by the gland. In the pig fetus Fenger (52) was able to detect storage of iodine in the thyroid in a 70-day stage, and it was also detected in 3-month fetuses of cattle and sheep. Hogben and Crew (71) were able to cause metamorphosis in axolotyls by feeding thyroids from 3-month sheep fetuses and 4-month cattle fetuses, at which time vesicles of the gland were beginning to appear and colloid droplets were evident. Rumph and Smith (144) found that extracts of the thyroid gland of pig fetuses introduced into hypophysectomized tadpoles were ineffective if taken from 70 mm. fetuses, when no follicles or colloid were evident, but extracts from glands of 90 mm. fetuses were decidedly effective in inducing metamorphosis of tadpoles; follicles were appearing and colloid droplets were evident. Rankin (135), in the pig, detected thyroxine and diiodotyrosine at the 90 mm. stage. According to Koneff and associates (97), concentration and storage of organic iodine occurs in the thyroid of fetal calves before the appearance of follicle formation. The glands from fetuses of 53 to 70 days' gestation —a period prior to appearance of intracellular colloid droplets or follicle formation—contained approximately 3 mg. per cent thyroxine and about 17 mg. per cent total iodine; this amount was at least ten times the amounts of these substances present in the liver. An increase in organic iodine concentration from 120 to 205 days of gestation accompanied gradual histologic development of thyroid follicles until in the eighth month of gestation it averaged 118 mg. per cent; "this increase in storage capacity of the thyroid was accompanied by a very marked rise of functional activity of the follicular cells."

In rat fetuses Gorbman and Evans (58) noted first storage of radioiodine

in the thyroid on the nineteenth day of gestation when a few follicles were just undergoing formation. Hall and Kaan (67) were able to demonstrate metamorphic effects in amphibian larvae from the thyroids of the fetal rat of 18 days, whereas those of 17-day fetuses were inactive; this provides evidence for the presence of secretions in the gland but not for release into the fetus with determinable effects. Thyroid secretions exercise an influence on the development of ossification centers in postnatal rats; thyroidectomy at birth delayed the appearance of ossification (7, 147) as did the injection of thiouracil at birth and later (117); introduction of thyroxine hastened the appearance of ossification centers occurring around postnatal day 10 (117). Weiss and Nobak (164) applied these methods to prenatal development by injections of thyroxine and of thiouracil into pregnant mothers. Thyroxine treatments failed to hasten ossification centers appearing in the normal 16-day fetus but thiouracil treatment of mothers did result in a delay in appearance of such centers. Although the authors maintain that thyroid secretion does influence differentiation of bone centers, it is impossible in this case to determine whether the source of secretions is the fetal rather than the maternal thyroid, with passage of the secretions through the placenta to the fetus.

These examples, and many uncited observations, illustrate the general principle that thyroid secretion in the developing young does occur and is closely correlated with the first appearance of definite follicles and the earliest appearance of colloid secretion. Less numerous, however, are demonstrations that such secretion plays any role in development. Recent studies on chick embryos by Wheeler and Hoffman (168, 169) do indicate an influence of thyroid activity and suggest the development of the reciprocal influences between thyroid and pituitary that are believed later to play a role in the regulation of production of such secretions.

The evidence suggests that normally thyroid hormone is deposited in the egg and that it influences development and the time of hatching; there appears to be established a normal reciprocal influence between the pituitary gland and the thyroid, such as can be shown in posthatching ages. Thus thiourea injected into developing eggs may retard hatching by as long as a week, but simultaneous introduction of thyroxine along with thiourea appears to neutralize the effect of the latter (65). Again, Wheeler and Hoffman (168, 169) fed thyroprotein to hens and obtained eggs requiring several hours longer to hatch than those from the same hens on a normal diet, due presumably to thyroid inactivation of the hen and a lesser deposition of thyroid hormone in the egg. In the chicks, developing presumably with a lowered thyroid hormone content in the egg, the thyroids at hatching were almost double the size of those from the normal chick; this effect is believed to depend upon an increased secretion of thyrotropin

by their pituitary glands (uninhibited by thyroid hormone), which caused an increase in size of their own thyroid glands. Andrews and Schnitzler (3) had earlier shown that eggs from hens fed thiouracil developed into chicks with a reduced metabolic rate, and thyroid gland weights of almost double those of normal controls. Further investigations will be required to clarify the mechanism that is operative under these conditions but the evidence appears substantial that, at least during later embryonic life in the developing chick, thyroid function does occur and that the hormone exerts an influence upon the embryonic pituitary gland.

Willier (173) demonstrated that the developing chick embryo is influenced by thyroid secretions when such are introduced. Adult thyroid gland tissue transferred to the chorio-allantoic membrane of an embryo caused the host chicks to be greatly emaciated, and to show a reduction in size of internal organs, including the host's own thyroid gland. These observations do not entirely support the above suggestion of a reciprocal pituitary-thyroid relationship, involving a functional control of their secretions, unless one considers that the chorio-allantoic thyroid graft was autonomous and did not require the stimulation by a thyrotropic substance from the pituitary; the graft should have been as inactive as the host's own thyroid. The adult tissue may have retained sufficient capacity for the thyroid graft to continue a grade of secretion sufficient to provide a continuous amount of thyroid hormone to the host embryo, thus exerting the effects observed.

It follows, therefore, that the thyroid gland produces a hormone during relatively early stages of development that can be demonstrated by various means. It is also well known that developmental stages of amphibian larvae are greatly influenced by the thyroid hormone, as indicated by the striking changes during metamorphosis. There appears to be a decided lack of evidence in mammals for marked influences upon early development due to secretions from the thyroid gland, although the well known condition of cretinism, or lack of growth due to absence of thyroid hormone, becomes evident shortly after birth in thyroidectomized rats, rabbits and other mammalian young.

IV. SUPRARENALS

In turning to the suprarenal glands for evidence of early secretion or function of these glands during embryonic development, three more or less different tissues or possible sources of secretion, present themselves for detailed consideration; these include a) the adrenal medulla (chromaffin cells) or the source of formation of epinephrine or adrenalin, b) the X-zone (also called boundary zone, fetal cortex, androgenic tissue, accessory cortical tissue) which has been considered by some as the source of the

sex-hormone-like secretions, and c) the generalized cortex to which many different types of secretion have been attributed.

A. Medulla

An examination of the embryonic adrenal for the well known chromaffin cell reaction of medullary elements, and for the presence of epinephrine, constitutes the major procedures in investigations of this part of the gland. It has been assumed that the color reactions displayed by medullary cells from treatment with various chromium salts is a valid indication of the presence of epinephrine but pharmacologic reactions are frequently employed to denote the presence of these secretions.

In the chick embryo a chromic response was demonstrated (118) as early as the eighth day of incubation; and whereas emulsions of the glands from the 7-day embryo were negative, those from 8-day or older embryos exerted depressing effects upon contraction of intestinal musculature; Lutz and Case (104) had previously reported the presence of epinephrine from adrenals of the 11-day incubation stage.

Among mammals, several studies have made known an early chromaffin response as well as the presence of epinephrine in the fetal adrenals. Thus, in the mouse, Howard-Miller (73) reported a distinct chromaffin response by the fifteenth day of development when medullary cells were just being incorporated in the cortex. Weymann (166) found good chromaffin responses in the fetal pig adrenal from the 45 mm. stage and later, and Fenger (52) reported epinephrine in the fetuses of cattle, sheep and hogs about the sixth week of gestation. In the human fetus, Lewis (98) failed to find epinephrine in the adrenals until about the time of birth, but Keene and Hewer (92) were able to find poor chromophile reactions by the twenty-fifth week of gestation and the presence of small amounts of epinephrine about the eighteenth week.

These, and other investigations, although making it apparent that the chromophile reactions in cells of the medulla and the presence of epinephrine in the tissues, occur at an early stage of development provide no evidence for a functional role of the hormone in development or differentiation of tissues. Indeed, the removal of the medulla of an adult animal has failed to so impair it (except perhaps when placed under stress conditions) as to make it clear that epinephrine plays a very general role in the organism. Consequently it has not been shown that a secretion from the adrenal medulla of an embryo plays any role during the process of development.

B. X-Zone (androgenic zone)

The fetal adrenal of man has long been known to be disproportionately

large, and marked regression occurs in the total organ beginning immediately with birth. Scammon (145) has shown graphically a) the rapid increase in adrenal weight from about the third fetal month until parturition, when it attains a weight of 9 grams; b) a reduction in weight within the first two postnatal weeks to 3.8 grams; and c) a slow juvenile growth that restores the birth weight of the organ at about the fifteenth year of life. Elliott and Armour (47) demonstrated that from the third fetal month until birth a prominently hypertrophied "fetal cortex" was present between the true thin peripheral cortex and the medulla; this "fetal cortex" showed definite involution changes immediately after birth and by the end of the first postnatal year the temporarily differentiated tissue had apparently been completely removed.

In the postnatal mouse Masui and Tamura (107) noted a transiently persisting inner cortical zone in the adrenal and this structure has been carefully studied by Howard (74 to 78). Designated as "X zone" by Howard, this temporary differentiation in the mouse adrenal cortex appears about the third postnatal week, disappears in males at sexual maturity and in females usually with the first pregnancy. Howard suggested the X-zone was equivalent to the fetal cortex in man (*see* also Davies (45) for the cat and Hill (70) for primates) and it has been referred to, therefore, as "fetal cortex," "boundary zone," "embryonic cortex," "X-zone," and as "androgenic zone." Considerable evidence has been presented in the mouse and rat for the secretion by this tissue of androgenic substances that are partially responsible for early postnatal secretory differentiation of the accessory reproductive organs of the male (Howard (74–78); Price (124–129); Burrill and Greene (25–28)). Grollman (64) would include under "androgenic tissue" at least much of the accessory cortical tissue, along with the X-zone, and would attribute the origin of adrenal masculinizing tumors in women to residual groups of androgenic cells that later become activated and undergo hypertrophy. Davidson and Moon (44) and Davidson (43) also point out that in early postnatal rats the adrenal cortex can be markedly stimulated to increased size and to the secretion of an andromimetic substance that affects male accessory reproductive organs; total weight of combined prostate and seminal vesicles was doubled and histologic changes in the prostate suggested an androgenic function, although structural changes in seminal vesicles were minor; a stage of secretory granules in seminal vesicle epithelium was not attained. Howard (77), however, doubts the primary function of the X-zone as a masculinizzing tissue and considers that androgenic capacity probably resides in the general cortex rather than exclusively in the temporarily differentiated zone; she points out (78) that castrated-adrenalectomized mice have normal prostates and seminal vesicles if they are maintained in good con-

dition by appropriate diet or by treatment with desoxycorticosterone, which has little androgenic effect in mice.

Since Broster and Vines (15) noted that masculinizing adrenal tumors usually revealed the "fuchsinophile reaction" and that a similar response to the stain was seen in human fetal cortex from about the eleventh to the sixteenth week, there has been considerable speculation relative to the possible contribution of an adrenal androgenic substance to the developing human fetus, and the possible role it might play in sex differentiation. Such speculations are not thus far sufficiently supported by tangible evidence to more than suggest a possible embryonic function for this portion of the adrenal. The human fetal cortex may represent merely an early local response of the developing adrenal to some hormonal substance present during pregnancy. Studies on the degeneration of the fetal cortex (8, 100, 158) reveal a beginning of the process as parturition becomes imminent and a precipitous involution of the cortex immediately after birth. At birth, 85 per cent of the cortex is represented by this tissue but within two weeks its sudden recession accounts for a 50 per cent reduction in volume of the whole gland. The degenerative processes are distinctly associated with cessation of the pregnant state and not with the state of development of any organ. Premature (7½-month) offspring surviving thirty days exhibit involution changes typical of those occurring at full term, hence the response is probably due to the absence of some substance available during the pregnant state.

It is known that other embryonic organs, such as the uterus and prostate gland, show regression in size and some histologic change following parturition. In the mouse, the X-zone comes into evidence only after birth and hence exhibits a different developmental relationship to the human fetal cortex. Martin (105) believes its persistence depends upon the pituitary gonadotropic substance and that gonadal hormones suppress it. Carnes (31) was unable to detect androgenic or estrogenic properties from extracts of human fetal and newborn adrenals, of from 18 to 40 weeks' gestation, placed directly on the comb of day-old chicks, or injected into immature female rats. The extracts of Gersh and Grollman (57) made from the fetal pig cortex, from fetal and newborn human cortex, and from X-zone-bearing adrenals of mice failed to exert an androgenic effect when injected into capons or castrated rats. In an attempt to determine whether the fetal cortex secreted glucocorticoids, "those adrenal hormones which promote the conversion of body protein to carbohydrate," Venning and co-workers (163) assayed urine from premature and full term human fetuses. The glucocorticoids in the urine of the two groups failed to show quantitative differences; furthermore, since the amounts of these substances in the urine tended to increase with age in both groups, at a time when fetal cor-

tex involution was greatest, there was no suggestion that the fetal cortex of the premature or newborn was elaborating the substances.

Thus, despite the somewhat dramatic character of the fetal cortex, its suggested similarity to the X-zone of a few postnatal mammals, and the idea that this portion of the adrenal secretes substances important in the development of the reproductive system, it would appear that up to the present time there is insufficient evidence to permit assigning to this portion of the adrenal a functional role during embryonic development.

C. Generalized cortex

Aside from the conspicuous behavior of the X-zone of the human fetal adrenal, and perhaps of all primates (70), there remains to be considered the generalized cortex which, in the absence of an X-zone, would include all the cortical tissue. Many observations or the results of experiment have prompted free speculation and suggestions of function for the cortex during embryonic development. Conditions that are definitely linked with the postnatal adrenal function, such as maintenance of life and proper growth, disturbances in blood electrolytes, carbohydrate metabolism and muscular weakness, have not been applied to the study of embryos. Much speculation has centered on the possible modifications of the reproductive system by unusual secretion from the fetal adrenal, especially when these have been associated with congenital disorders such as the various grades of hermaphrodism. Linking cause and effect in such conditions is a hazardous procedure and whereas one recognizes the evidence which links postnatal associations of the cortex and the genital system, such an association during embryonic life is not so clearly recognized.

Adrenalectomy of pregnant females has been found to be less effective immediately than in nonpregnant animals and Grollman (64) suggests an explanation for the delayed death of the mother on the assumption that cortical hormone produced by the fetus is transported through the placenta to the maternal organism. If such an explanation were tenable, these results would provide a means for determining at what period in embryonic development the fetal cortex began its secretion. However, when broadly considered, the evidence fails to support such an interpretation.

Rogoff and Stewart (141) clearly established a protective influence against adrenalectomy during pregnancy and following parturition in dogs; and although Corey (37) failed to confirm these results when cats were used, the later work of Collings (35) demonstrated a similarity in response in pregnant cats and dogs. Firor and Grollman (53), in rats, found essentially a doubling of the survival period when adrenalectomy was performed during pregnancy. However, careful examination of the data of Rogoff and Stewart reveals that very early pregnancy provided greater protection than

late pregnancy; that marked protection was afforded by proestrus and estrus not followed by pregnancy, (hence in the absence of fetal adrenals); and that protection was provided by impregnation one week prior to adrenalectomy and therefore prior to development of fetal adrenals. These authors pointed out that all protection noted by them could not have depended upon fetal cortex secretion and they made the passing suggestion that the corpus luteum might be involved. Corey pointed to death of pregnant mothers from adrenal insufficiency at a period during which four full term fetuses were present. With the rat, Firor and Grollman noted that protection against adrenal insufficiency extended up to the sixteenth day post partum. It thus appears more probable that the protection afforded during pregnancy and in association with estrus or in postpartum periods, depends upon secretions from the corpus luteum of the ovary rather than upon fetal secreted substances. Subsequent investigations in studies on pseudopregnancy, on luteinization of the ovary, and on injections of progesterone leave little doubt that the protective mechanism involved during pregnancy or heat periods is due to secretions from the corpus luteum and not from fetal adrenal cortical secretion (35, 41, 56 146).

It has been maintained that young postnatal animals succumb to adrenalectomy more rapidly than do older ones; Firor and Grollman (53) and Sisson and March (148) found that rats adrenalectomized at 20 days of age survived on an average for seventeen days. From these and other similar experiments, it becomes evident that cortical function in the prolongation of life expresses itself shortly after birth. Whether similar secretions play a role during embryonic life has not been clearly established. It should be pointed out that Tobin (159) was able to destroy both adrenals of fetal rats on the seventeenth day of pregnancy by the use of fine-point cautery and noted that survival and essentially normal growth was maintained up to removal from the uterus on the twenty-third day; thus body weight, thyroids, gonads and accessory reproductive organs were normal, although parathyroids appeared to exhibit hypertrophy. Tobin believes the embryonic adrenal does not perform the function of the postnatal adrenal.

Thus, as one considers broadly the evidence for adrenal function during embryonic life, it becomes apparent that epinephrine is present in the gland and can be extracted but the evidence for any function, or even for the release of this secretion from the gland tissue into the developing organism, appears to be lacking. Furthermore, evidence for a definite function on the part of the X-zone, or the more generalized cortex, appears to be lacking despite the quantity of speculation on the subject. This is not to say that the embryonic adrenal has no function, but merely to express the opinion that a definite functional role is yet to be established.

V. PANCREAS

The literature concerned with possible evidence for hormone production, or for a functional role during embryonic life, for the pancreas or parathyroid gland is so scanty and controversial as to make rather futile an attempt to evaluate it. A few considerations only will be given to the pancreas to indicate the general problem.

The problem is well introduced by the experiments in 1911 of Carlson and Drennan (30) who depancreatized adult dogs and noted that the ensuing diabetes was much less severe during pregnancy than in nonpregnant dogs; in the pregnant bitch pancreas removal at an early stage had more severe effects than when removed later in pregnancy. Following parturition typical diabetes developed. This evidence suggested that when the fetal pancreas was well developed, it secreted sufficient insulin to pass through the placenta and protect the mother from the effects of loss of her pancreas. The experiment was repeated with some modification in 1936 by Cuthbert, Ivy, Isaacs and Gray (40) with confirmatory results, but they noted also amelioration of diabetic symptoms in the mother by the nursing of pups. The authors lean more to the interpretation that late pregnancy and lactation provide a stronger call for maternal sugar and its greater utilization under these conditions, rather than that fetal secreted insulin accounts for the reduction of glycosuria in the mother. It should be mentioned, at least, that great controversy prevails relative to the permeability of the placenta for insulin (38, 79, 90, 155, 181).

The study of the histologic differentiation of the pancreas by Hard (68) showed that beta cells of islet tissue, presumably the insulin-secreting cells, were the only type to differentiate during embryonic development in the rat; alpha cells do not appear until early postparturitional life. Beta granules were first distinguished on the nineteenth day and this indication suggested the cells were capable of considerable insulin secretion. However, Hard leans to the interpretation that (if insulin is required by the fetus) the insulin is provided by the mother rather than by the fetus, and that probably beta cells do not release insulin until postparturitional stages.

The fact that differentiation of islet cells in some mammals occurs prior to differentiation of acinar tissues and the formation of enzymatic secretions suggests the possibility of secretory activity (122). Banting and Best (6) extracted fetal calf's pancreas of 5 months' development and obtained insulin in proportionately greater amounts than from adult pancreas.

This brief mention of some of the problems associated with the question of the time of effective secretion of insulin by the fetal pancreas provides strong evidence for an early fetal production of insulin that can be extracted

from the fetal pancreas. However, it does not prove that insulin is liberated from the fetal pancreas to play a role in physiologic regulatory mechanisms in either the fetus or the mother.

VI. GONADS

The question of the function of gonads during embryonic development is a problem presenting difficulties somewhat similar to those associated with other organs of internal secretion and involves the problem of the stage in development when these organs produce and release an internal secretion in effective amounts; also whether such secretions are present during development and play any role in the process. Although the gonadal problem has received considerably more attention and active experimentation than that of other hormone-secreting organs during embryonic development, one does not find a more general unanimity of opinion of observed phenomena; and at the onset one must admit to much diversity in interpretation of observations. Clarification and final general agreement among investigators as to the meaning of observed phenomena are yet to be attained.

The dawn of the present century marks the first real interest and consideration of the general problem, for at that period Bouin and Ancel (11, 12) on the basis of brilliant studies on the interstitial cells of the testis of embryos, adults and experimentally modified sex glands of mammals, suggested that hormone secretions from the embryonic interstitial cells were responsible for the control of the development and differentiation of sex cells and the organs of the reproductive system. Very substantial evidence of a quite convincing nature was contributed by Lillie (101–103) and Keller and Tandler (93) through their studies on the freemartin, in which case nature performed a most important experiment. These studies laid the foundation for the generally accepted notion that very early in development embryonic sex glands secrete sex hormones which are responsible for the differentiation of the two sexes from an indifferent type prevailing in each embryo: each contains the basic building stones for the production of either sex type. Special attention to this important investigation, therefore, is merited.

Briefly, the development of twins in cattle involves the very special condition of fusion between the embryonic membranes of the elongated chorionic vesicles. Not only is there fusion between the chorionic and allantoic membranes of the two individuals but of greater importance is the fusion of the chorioallantoic blood vessels of the twins such as to permit an admixture of blood between the twins. Only in case the twins are of different sex do unusual events occur and these apply to the female; development of her reproductive system suffers severe modification.

In this female twin of a heterosexual pair, known as a "freemartin," either an ovarian cortex does not develop, or if it starts development it undergoes degeneration, and the medulla of the ovary proceeds in development as a testis; the female duct system (Müllerian ducts) degenerates rather than differentiates into oviduct, uterus and part of the vagina; the embryonic male duct system (Wolffian ducts) rather than degenerating as is the normal course in females is preserved and produces a typical male reproductive system. The genetic female, therefore, is modified during development and becomes much more like a male individual, although the external genitalia usually remain female in type.

The essence of Lillie's explanation of this most unusual development in the female reproductive system is essentially the following. Testis differentiation, being somewhat more precocious than ovarian development, permits secretion of a male sex hormone that passes by way of the conjoined blood vessels into the female partner, and the modification of female reproductive development, therefore, is caused by the internal secretion, or sex hormone, from the testis. Steinach had earlier developed the notion of sex hormone antagonism from his studies on males and females of adult animals; he endowed sex hormones with the double function of stimulation and inhibition in which masculine qualities in the organism were stimulated by male sex hormones, and at the same time feminine qualities were inhibited. The observed conditions found in the developing freemartin appeared to be explained logically by a similar concept. Although no evidence was presented by the freemartin for secretions from the ovary, on account of earlier action by male hormones in causing ovarian degeneration, it was logically presumed that ovarian secretions would have similar consequences if introduced into developing males.

Upon these observations and interpretations of conditions found in the freemartin rests our present, most generally accepted, idea that each genetic sex produces a gonad of common type as well as a double sex duct system in the embryo (the indifferent stage), and that as the gonad differentiates into testis or ovary it secretes a sex hormone (male or female as the case may be) which is responsible for stimulating further development and differentiation of the homologous ducts and other proper accessory reproductive organs, whereas at the same time it inhibits development of the components of the opposite sex. Thus normal sex differentiation would be under the control of gonadal secreted sex hormones in the embryo. This theory has become generally known as the dihormonic theory of sexual differentiation. Lillie, himself, insisted that the freemartin interpretation, logical as it was, should be proved by experimental means. The attempts to obtain satisfactory experimental evidence during the last thirty-four years have involved the efforts of numerous investigators, located in several

countries, who have utilized members of all classes of vertebrates from fish to mammal for experimental materials; and at the present time many diversities of opinion exist as to the meaning of the vast accumulation of facts and how they should be interpreted.

A. Evidence for sex hormone secretion by living tissues

Prior to the availability of the steroidal sex hormones, extracted from natural sources or prepared synthetically, that have shown such capabilities of substituting for the true gonadal secretions in juvenile and adult vertebrates, experimental procedures depended usually upon some form of surgical intervention—ablation or transplantation of sex glands, as well as parabiotic union between the different sexes. Such procedures in different species of amphibians yielded interesting results in the experiments of Burns (17, 18) Witschi (183), Humphrey (82–84), Ponse (123) and other investigators. Thus in many cases the parabiotic union of heterosexual pairs of urodeles frequently was associated with modifications of the ovary by the testis; in frog larvae the influence was largely restricted to ovaries in close proximity to testes, whereas in the toad the testis components exerted an influence over the ovarian portion only when in direct contact with it. The effects have been interpreted as being due to the influence of secretions from the gonadal components, "medullarin" being produced in the medullary portion of the gonad and "cortexin" in the cortical portion of the gonad. Secretions from the genetic testis usually exert a predominating influence over the ovary, inhibiting the cortical portion and permitting the medullary components to develop as testis. Under certain conditions, however, the reverse was found when cortexin inhibited the medullary portion and the gonad in the male developed as an ovary.

Similar changes have been noted by Humphrey to follow transplantation of the primordial sex gland into an opposite sexed host and, again, it is most frequent that the developing ovary is modified by the testis, in some instances so completely that the modified ovary may produce spermatozoa (82, 83).

These modifications of gonads obtained in larval amphibians as experimental subjects have some similarity to the gonadal modification described in the freemartin. However, the influence is one operating between the two components of the gonad alone and does not affect the duct system. In fact, both Witschi and Humphrey feel assured that the active substances are entirely separate and different from the true sex hormones that come into action at a later period in development. Rather than being steroidal substances as are the natural sex hormones, Witschi, especially, believes that medullarin and cortexin are protein-like substances which both investigators regard as inductor substances.

Transplantation of gonads has likewise been performed in developing chicks. The original work of Minoura (108) in which gonad fragments were transplanted onto the chorio-allantoic membrane of the chick, the results of which were reported as showing definite modifications in hosts of opposite sex, has been criticized vigorously by Greenwood (63) and by Willier and Yuh (176); extensive experiments by Willier (174) failed to elicit any modifications that were not also produced by non-gonadal tissue transplants. At the present time, therefore, it is generally accepted that gonad grafts on the chorio-allantoic membrane do not exert any modification in the host reproductive development, nor does the intact host gonad exert modifications on the gonad graft tissue. Wolff (184), however, transplanted gonads from chick embryos of 6 to 11 days' incubation into the coelom of 48–52-hour host embryos and reported modifications of the developing reproductive tract of opposite sexed hosts. Ovarian transplants in the coelom of male embryos were reported to stimulate cortical development on host testes, and testis grafts in the coelom of female embryos caused degeneration of part of the female ducts. Wolff regards the action as due to substances secreted by the gonad graft that exerted only a local effect through diffusion, but since these effects were similar to some of the effects that follow the injection of an androgenic or estrogenic substance into the developing egg, he regards the secretions as steroidal sex hormones of a nature identical with, or very similar to, the adult sex hormones secreted by the gonads.

The embryos of most mammals are somewhat less accessible for transplantation than those of other vertebrates and the report of Jost (88) on the rabbit appears to represent the only successful efforts thus far described. Earlier, however, a reverse type of procedure aiming at the same set of conditions was attempted by Buyse (29) and by Moore and Price (115); Buyse transplanted the posterior portion of the rat embryo, prior to sex differentiation, underneath the kidney capsule of adult intact hosts, and Moore and Price dissected the embryonic reproductive system from rats as early as the sex could be determined, transplanting these subcutaneously into intact and castrated hosts of 4 to 30 days of age. Whereas in each case the developing reproductive system had the possibility of continued development in the hormonal environment of the host, often of opposite sex to the developing transplant, in neither investigation was evidence obtained for modifications of the transplanted developing embryonic tissue by host hormones.

Jost (88) successfully transplanted gonads into fetal rabbits in utero as early as day 20 of pregnancy at a period when both sex ducts were present, recovering the fetus for examination on the twenty-eighth day just prior to expected delivery. Gonad transplants to subcutaneous positions in the same or opposite sex failed to produce any modification in development of

the host reproductive system; testis grafts transplanted into the coelom of normal female hosts on the twenty-third day of development exerted no modifying effects on the female reproductive system, even when the graft was in contact with an ovary; but in one case a 21-day fetal testis transplanted into the coelom of a female, ovariectomized on day 20, was described as having some effect. Examination of the host female some days after transplantation showed the absence of an oviduct on one side, although the uterus was present, and the presence of a Wolffian duct throughout its length; the opposite side revealed an intact Müllerian duct and portions of a Wolffian duct. Jost regards this case as demonstrating an inhibiting effect of testis-secreted hormone on the host oviduct of one side and a stimulating effect on the Wolffian duct, stronger on the side of the transplant than on the opposite.

In addition to the transplantation experiments in mammals other observations may be mentioned of conditions in which it is to be expected that hormones secreted by living tissues, in contrast to chemically prepared pure steroidal hormones, might be present and very well play a role in the development of tissues or organs. Hughes (80, 81) described several cases in which fusion of chorionic vesicles had occurred in heterosexual pairs of pig fetuses. Some modifications in the developing female reproductive system were noted that were quite similar to those described for the freemartin. The modifications were often less radical than that seen in the fetal calf, especially as regards the ovary; in the pig an active germinal epithelium and cortex was present in some cases. On the whole, however, there was fair agreement with findings reported in the freemartin. Two other species in which definite blood vessel union was reported between heterosexual pairs failed to show modifications. Thus Wislocki and Hamlett (182) described one pair of heterosexual twins in the cat in which well developed blood vessel fusion was present and the reproductive system of both sexes was unmodified. Wislocki (180) points out that in the marmoset, twins are of frequent occurrence and heterosexual pairs were observed in which strong blood vessel connections existed, yet neither of the pairs revealed departures from a normal reproductive system. It should be pointed out also that Jost (88) successfully united rabbit fetuses of opposite sex by an approximation of the thighs on the twenty-second and twenty-third days of pregnancy. Fusion and blood vessel connection between the pair was proved by the passage of fluorescein from one to the other, when at autopsy this substance was injected into one of the members of the pair. Despite the blood interchange between the heterosexual pairs, Jost failed to find evidence that hormones from either sex exerted any modifying influence upon the reproductive system of the opposite member.

Thus when one considers the available evidence for effects from secreted

hormone from the embryo upon the developing reproductive system, the original evidence derived from a study of heterosexual twins of cattle receives support to a fair degree from heterosexual twins in the pig but not from such pairs in the cat or the marmoset; inadequacy in the amount of available material for observations in the two latter species thus far, must be admitted. Jost's parabiosis experiments in heterosexual pairs of rabbit fetuses fail to provide substantial evidence for effects from embryonic sex hormones. The various experiments on transplantation of gonads, or parabiosis, or the transplantation of developing reproductive tissues into strange hormonal environments in amphibians, birds, or mammals fail to provide clear evidence for the influence of gonadal secreted sex hormones, except for the intergonadal influences described for the amphibia; the latter phenomenon is interpreted by the investigators describing the results as being due not to the recognized steroidal sex hormones but to protein-like inductor substances. It becomes evident, therefore, that results from experimentation thus far conducted leave much to be desired in support of the dihormonic theory, the original evidence for which was supplied by the freemartin. One may well keep in mind the possibility that development in cattle fetuses, and to some extent in pigs, may present some special unknown peculiarity that does not apply in other animal forms, but such a postulation requires considerably more evidence than that presently available to set these aside in a category that differs from conditions prevailing in development of other vertebrate forms.

B. Steroid chemical substances

It was originally a general hope that when active preparations of male and female sex hormones became available for use it would become a simple matter to confirm by experiment the basic findings that nature had earlier provided in the freemartin condition. During progress in studies on the extraction and purification of these sex hormone substances from natural sources, their chemical analysis, and finally the synthesis of many of them, numerous experiments have been executed to study their effects upon the reproductive systems of developing embryos. In another place (114) more detailed citations have been given to these experiments than will be entered here, but it may be stated that numerous pure chemical androgenic and estrogenic substances, as well as unpurified extracts, have been employed to study their capabilities of modifying the developing reproductive system, using members of all the classes of vertebrates as experimental subjects. One naturally questions whether any preparation utilized can be rated the equivalent of any possible natural hormone that is secreted by embryonic gonads and is supposed to operate during normal development, but Wolff (184) and Jost (88) argue in the affirmative; they

consider that embryonic sex gland secretions are steroidal in nature and identical with, or similar to, the adult sex gland secretions, and that certain of the chemical androgens and estrogens are complete substitutes for them.

A general statement relative to the effects of these chemical androgens and estrogens on developing fish or amphibians should reveal that frequently treatment with androgenic substances is followed by a reversal of females toward male development, and with estrogenic substances a reversal of males toward females; but also that different species may show a reverse action ("paradoxical effect"), or again that steroidal substances having little androgenic or estrogenic properties by usual tests may be effective in causing sex reversals. Among anuran larvae, sex reversals may be effected easily by the proper hormonal treatment, but urodele larvae fail to give similar responses and may show little or no effects from the treatments with steroidal hormones (Mintz, 109). It should be pointed out also that sex reversals in amphibia may be induced by treatments of developing young by other means than with steroidal hormones, *i.e.*, by changes in temperature of the medium in which they develop, overripeness of eggs, etc. (Witschi, 183).

Development of the embryonic reproductive system in birds may be highly modified by the introduction of pure chemical hormones into the developing egg (99, 175, 184, 185). Several androgenic substances of slightly different chemical composition exert masculinizing influences in genetic females but the effects are not always of the same character. Investigators have emphasized perhaps most strongly the inhibition of cortical development on the ovary, frequently accompanied by increased size of the medulla and the inhibition of the left female duct, although in many instances the right duct may be present and larger than normal. Some androgens (androsterone) exert decidedly feminizing actions such as the retention and growth of female ducts in the genetic male embryo. Estrogenic substances likewise provoke modifications in sex development usually regarded as feminizing reactions. When administered to genetic male embryos the left testis in particular, and sometimes the right, may produce a conspicuous development of cortex along with a reduction of the medullary portion; the testes assume, therefore, a rather decided general morphology of the ovary. In males, also, such treatments may result in the retention of one or both of the female ducts but it is not usual that the male duct system is inhibited by estrogens. Some of the findings reported by different investigators support the dihormonic theory, whereas others fail to do so. Since, however, the exposure of developing eggs to non-hormonal influences may also render striking modifications in the developing reproductive system, one may well question whether the various modications occurring after hormone treatments are specific responses to the

hormone per se or to modifications in the usual physiology of development that might be induced by many different treatments. In this connection Willier and Yuh (176) reported abnormalities in the reproductive system of late incubation stage chick embryos that had been subjected to a) ordinary normal incubation, b) transplantation of tissues of gonadal or non-gonadal origin, c) the operative technic without transplantation, d) altered humidity. Furthermore, the abnormalities were entirely similar to modifications that have been attributed to the effects of androgens and estrogens. Thus, in males, these investigators reported 1) the presence of oviducts, in whole or in part, on right, left or both sides; these may be full length ducts, with an ostium and even with a definite shell gland development. 2) The testes may be modified sufficiently so that they closely resemble the ovary in embryos of similar ages. In females, the right oviduct may be retained in its entirety, as well as the right ovary of large size, with or without, a conspicuous cortex. Since this variety of atypical conditions is thus obtained without the use of steroidal hormones, it is difficult to see the justification of referring similar ones to a specific action of the hormones when these have been administered.

Finally to be considered here is the evidence that bears upon the concept of a hormonal control of sex differentiation in normal embryonic development that has been derived from treatments of mammalian embryos with various of the pure chemical hormones. Is it indicated that normal sex differentiation depends upon hormones secreted by the developing gonad?

A large number of facts have accumulated from observations on the developing young of mammals subjected to treatments with pure chemical hormones, which have been regarded of the greatest importance in their bearing upon the problem. It is to be emphasized that little controversy exists relative to observations contributed by different investigators; there is decided controversy, however, relative to the interpretations of the observations and their proper assessment in support of the dihormonic theory, or modifications of this theory called forth to explain certain observations. Species of mammals employed in these studies include the rat (59–62, 110), the mouse (136, 137, 160, 161), guinea pig (42), hamster (16, 170, 171), rabbit (87), monkey (162), man (46) and opossum (19–24, 111–114).

Greene, Burrill and Ivy have utilized many different pure chemical hormones for treatment of pregnant rats, the goal of the treatment being an attempt to subject the developing young in utero to these substances by transmission through the placenta from mother to young. Observations were made on young, principally at birth and later postnatal stages, to determine in what manner the differentiation of the reproductive tract may have been modified by the hormones. Confusing elements were en-

countered almost immediately, as was emphasized by Moore (110), in that subcutaneous administration of pure chemical androgens was injurious to the placenta; daily dosages of from 0.5 mg. to 5.0 mg. given to the mother by subcutaneous injection frequently resulted in the death of from 40 per cent to 100 per cent of fetuses by the twenty-first day of pregnancy. Since many kinds of deviations from normal embryonic development are known to follow many types of experimental treatment of developing vertebrates, as well as invertebrates, it immediately becomes a question whether atypical development could be attributed to hormonal activity when the modifications were found in the reproductive or other systems. Could not interference with normal physiologic processes, instead of the hormone action as we usually refer to this term, be responsible for the deviations, especially in such rapidly changing parts as the reproductive system between the fifteenth and twenty-first days of pregnancy in the rat? This appears to be a pertinent question in connection with an evaluation of the effects following treatments with chemical hormones now under consideration.

The first important observations of Greene, Burrill and Ivy on modification of the reproductive system during development by treatments of the mother with androgens, applied to the female offspring; here, it has been hoped, would be a test for the hormone concept derived from freemartin studies. It was noted (59) that female offspring possessed a well differentiated male reproductive system (epididymis, ductus deferens, prostate gland, seminal vesicles, etc.) in addition to the female tract. This finding has been regarded by many as strong evidence for the correctness of the hormone theory, since there is stimulation of homologous components (Wolffian duct in female). Evidence for the inhibition of heterologous characters (female components) is less satisfying, however, since the inhibitions cited by these authors were largely limited to an absence of the terminal vagina and inhibition of the ovarian capsule; there was not an inhibition of the ovary, oviduct or uterus (all of which occurred in the freemartin); hence it is obvious that only partial confirmation of the dihormonic theory was apparent. Further difficulties were encountered in evaluating the effects of estrogens introduced into the mother. Following the treatment, female offspring were obtained that likewise showed an unusual retention of the male duct system, and there was similar inhibitory action to androgen treatments on the ovarian capsule and distal vagina; stimulation of the uterus and nipples was present (60). It is obvious, therefore, that in the usual usage of the terms, the female hormone had stimulated the opposite sex characters (Wolffian ducts) and had inhibited homologous characters (terminal vagina, ovarian capsule); the results were designated "paradoxical."

Since simplification of experimental procedures is desirable, the utilization of an animal such as the opossum offered some promise from the fact that it is born in a sexually undifferentiated stage and the pouch young can be treated with hormones directly, instead of by way of the pregnant mother; the feature of a more leisurely progress in sex differentiation is also an asset. In pouch young opossums (gestation period 12 to 13 days) testes are identifiable on day 3; Müllerian ducts first appear at their anterior end on day 3 and reach the urogenital sinus on day 10; male and female systems are very definite by day 30 and the opposite set of sex ducts is largely gone. Thus, to perfect a comparable degree of sex differentiation, the opossum utilizes a period of approximately twenty-five to thirty days of postnatal life, in contrast to the rat, of a period of five to six days preceding parturition.

Burns (19–24) and Moore (111–114) have utilized the pouch young opossum for direct treatments with sex hormones. Whereas methods of treatment and total hormone dosage differed somewhat, the observations of the two investigators are to a large extent similar, although their respective interpretations differ. In general, androgenic treatment of females resulted in some stimulation in the male direction—Wolffian duct retention, prostate development (normal female opossums never have a prostate primordium) and penis formation. Inhibitory actions on female structures were not apparent, however, since the ovary was largely unaffected and the oviduct, uterus, and lateral vaginal canal, instead of suffering destruction, were actually precociously stimulated. In males treated with estrogens, some stimulation of Müllerian ducts and nipples was noted; again, however, inhibitions were largely absent, since testis development and the male duct system were essentially normal. The prostate area of the urogenital sinus was modified—inhibited in Burns' estimation but stimulated in an unusual manner according to Moore—with somewhat similar reactions on the phallus. Rather than being inhibited, the male sex ducts were stimulated by estrogens.

Burns maintains that sufficiently low doses of androgen may still stimulate male qualities without stimulation of the female structures, but the question of threshold responses cannot be neglected. The experience of Burns on the opossum, and a consideration of findings by other investigators, leads him to subscribe to the notion that the freemartin hypothesis has received final proof (24), whereas Moore (114) emphasizes the inadequacy of experimental results thus far obtained to substantiate the freemartin interpretation or the dihormonic theory of sex differentiation. It is fair to state that in sex differentiation in mammals, as indicated by reproductive tract development after chemical hormone treatment, no one has yet closely approached production of conditions that were presented by

the freemartin. A consideration of the aggregate of results obtained from treatment of young with chemical hormones leads Moore to believe that convincing evidence for secretion of sex hormones in embryonic gonads and effects from these on differentiation of the reproductive system, is yet to be obtained; his notion that the control of sex differentiation resides in the general genetic constitution of the organism rather than in the hormonal secretion of embryonic gonads receives further support in castration experiments reviewed below.

Any attempt to evaluate results obtained from introduction of chemical hormones either into the pregnant mother, for treatment of young in utero, or its direct application to embryos should take into consideration such modifications in reproductive tract development as have been shown to follow maintenance of pregnant mothers on a diet low in vitamin A. Wilson and Warkany (177) noted in the offspring of such treated mothers almost all the modifications in the reproductive tract that have been chronicled as effects from estrogenic or androgenic hormone. Thus in males, atypical conditions observed by them, and elsewhere cited as stimulation by an estrogen, included a) the presence of the female duct system in whole or in part; formerly attributed to an estrogenic inhibition were b) lack of testis descent, c) absence or inhibition of bulbo-urethral gland, seminal vesicles and male sex ducts, d) prostate gland inhibition, e) defective urogenital sinus, and f) hypospadias. In female fetuses, malformations formerly attributed to androgenic stimulation included a) presence of a male duct system, complete or in part; and as an androgen inhibition b) absence of Müllerian ducts in whole or in part, c) lack of distal vagina, d) lack of fusion of Müllerian ducts, e) defective urogenital sinus, and as estrogenic or androgenic stimulation f) metaplasia and keratinization of urogenital sinus epithelium. Since Wilson and Warkany did not utilize androgens or estrogens and obtained this array of modifications only as a result of lack of vitamin A in the mother's diet (179), the reader may be left the task of judging how adequate is the explanation for specific hormone actions when the same type of abnormality follows treatments of pregnant mothers with chemical hormones.

The question to be considered is whether the realization of such modifications from both types of treatment—steroidal hormone and deficiency of vitamin A—may not merely depend upon some modification in the rate or character of the fundamental processes of embryonic development and not represent an action of hormones in the usually accepted sense. Perhaps on such basis one may more easily understand the very limited, or absent response on the part of human or hamster fetuses (16, 46, 170, 171).

The utilization of steroidal sex hormones as treatment for developing

embryos has indeed been followed by profound effects upon the differentiation of the reproductive system, but the writer is unable to regard the array of modifications as strong supporting evidence for the notion that embryonic sex glands secrete hormones that control the differentiation of the reproductive tract. Thus it also follows that varied as are the results that follow administration of chemical hormones, they do not appear to give substantial evidence that in normal development embryonic hormone secretions control differentiation of the tissues of the reproductive tract.

C. Effects of embryonic castration in relation to sex differentiation

Further evidence bearing upon the dihormonal theory of sex differentiation has been sought through the removal of the embryonic gonad. Birds and mammals present considerable difficulties in gonad removal at early stages and the number of such investigations is not large. Conditions presented by the young opossum pouch young make it possible to remove gonads at an early stage of development, although they are no longer embryos; their progression in development is such, however, that the stage of differentiation is entirely equivalent to embryonic stages in placental mammals.

The writer succeeded in removing testes or ovaries from 20-day-old pouch young with survival possible for indefinite periods of time; the young were examined for study of the reproductive tract from one month to longer than three months after operation (Moore, 112). Twenty days in the pouch is the earliest age at which gonadectomy has thus far been reported. At this age the testes or ovaries are attached to the medial surface of a large and vascular mesonephros and both the male and female ducts in each sex are simple, straight, undifferentiated tubes still attached to the urogenital sinus, although at the posterior end the lumen of the heterologous duct may be occluded for a few sections (lumen of Müllerian duct in males, Wolffian duct in females). The prostate gland of males, that serves as a register for hormone action, makes first appearance on day 16 as a very small thickened knob of cells in the epithelium of the urogenital sinus, and by day 20 perhaps not more than a half-dozen such thickenings are present.

Following the removal of the testes on day 20, the prostate gland area continues to produce numerous additional knob-like thickenings and these, along with the few present at operation, continue to grow peripherally into mesenchymal tissue surrounding the future urethra; branching and rebranching carries the prostate gland up to slightly more than 100 days of age as a network of epithelial cords, solid for the most part. No differences in this prostate differentiation in extent, in character of development or in time required for its attainment was noted in the castrate and intact

male; the earliest appearance of detectable differences in the prostate of castrate and control intact males was at 130 days after birth (110 days postoperatively) when evidences for hormonal action in intact males was first apparent. A similar consequence follows in the case of Cowper's gland (142, 143); castration does not retard its development up to an age of approximately 100 days. The opossum does not have a seminal vesicle. Atrophy of the female ducts, present at operation, was as complete in the castrate as in the normal intact male.

In females subjected to ovarian removal, development of the Müllerian duct system proceeds as in intact females. Comparison of the female tract in ovariectomized and intact animals 100 days or older (approximately two months after operation) failed to demonstrate a detectable difference as a result of operation. At this period the formerly simple duct had differentiated into characteristic oviduct, enlarged uterus, constricted cervix and enlarged lateral vaginal canals. Wolffian duct atrophy was of similar grade in both types of females, only vestiges remaining at the anterior end.

The finding that removal of the developing gonad, at a time when both duct systems were present and each one contained a lumen, failed to interfere with the further differentiation of the reproductive structures provoked the conclusion that differentiation of the reproductive system could not have been controlled by gonadal secreted hormones (112).

The above interpretation has been criticized by Jost (88) with the suggestion that already at 20 days of pouch life the differentiating testis had secreted testis hormone that had imparted an influence which carried through to late developmental stages, which, once begun, proceeded without further aid of testis hormone. Jost has executed most excellent studies on the fetal rabbit that led to his modification of the hormonal theory to one of a monohormonal character different from the earlier monohormonal theory of Wiesner (172), constructed to account for his observations from the gonadectomy of postnatal rats.

Jost succeeded in removing rabbit fetuses from their embryonic membranes, performing gonadectomy, and replacing the fetuses inside their membranes without interrupting pregnancy. In males operated on during days 23 or 24 of pregnancy, with autopsy on day 28 just prior to expected parturition, castration failed to modify male sexual differentiation. As castrations were performed at still earlier ages, when both sex ducts were present, modifications in the male reproductive system were encountered. Gonadectomy on days 21 or 22 produced a retardation in development of prostate, ductus deferens and penis, along with interrupted remnants of the female ducts. When testes were removed on day 19, autopsy on day 28 revealed in some cases a lack of prostate gland, an absence of ductus deferens, female type external genitalia and the presence of an almost

completely normal female reproductive tract. Jost's interpretation of his observations involves the idea that secretions from the fetal testis have a primary action in the induction of the primordium of sex structures, after which the secretions play no further role in stimulation of growth or differentiation of reproductive structures; the male structures, such as ductus deferens, prostate and penis, failed to develop because of lack of testis secretions to induce a primordium for the structure or "fix" its further development, and the female tract developed because testis hormone is required to inhibit its development in the male. Unfortunately this interpretation of the necessity of testis secretions for induction of a prostate primordium does not provide an explanation for the presence in each normal developing female of a definite prostate anlage (86). In females, the removal of ovaries had a negligible effect upon reproductive tract development. Removal of ovaries at the earliest stage possible was followed by the further development of an almost completely normal female tract; Wolffian ducts had completely disappeared as in normal female development, presumably because of lack of testis hormone to "fix" its persistence.

Jost's interpretations introduce a concept largely new. In a manner similar to the earlier monohormonic theory of Wiesner, Jost would maintain that, despite the original genetic sex, all embryos would develop as females. To produce male development requires secretions from the embryonic testis to induce anlage or "fix" determination of male structures, and also requires testis secretions to banish the female structures. However, Jost's utilization of chemical androgens, considered by him to be similar to embryonic testis secretions, for treatment of female fetuses failed entirely to inhibit development of female structures in intact or in ovariectomized females (87, 88).

Thus, despite the exceedingly commendable experimentation and execution of difficult operative technics by Jost, it is yet difficult to accept the interpretations placed by himself on his own experiments, or upon the observations on opossum pouch young. Again, the writer considers that acceptable proof for the secretion of hormones by the embryonic gonad, and the action of these secretions in exercising a control over reproductive development, has not yet been presented; nor has the evidence presented by Moore from experiments on opossum pouch young been proved untenable.

D. Extraction of embryonic testes

Whereas extraction of fetal testes that may yield substances having an androgenic action would provide strong evidence for the secretion of androgenic substances and their presence in the gland, such a demonstration could not yet be accepted as proof, or entirely satisfactory evidence, that the secretions were released into the organism in effective amounts and

were instrumental in controlling differentiation of the embryonic reproductive system.

At least two investigations bear upon this aspect of the general problem. Womak and Koch (187) obtained androgenic substances from extraction of testes from fetal bulls shortly before parturition; such extracts proved capable of stimulating growth of the capon comb. However, the same investigators were unable to extract effective substances from the urine of postnatal bull calves. It is thus possible that like other glands of internal secretion (thyroid in larval amphibians, pituitary in immature mice), testis secretions may be present in the gland without being released in effective amounts in the organism. In any event secretions were not detected in the testis until a period long after the attainment of well differentiated male and female systems.

Atsumi (4, 5), working in Korea, carried out extractions of fetal calf testes and ovaries, fetal blood, and maternal blood, and tested the extracts in properly prepared mice or rats; by proper test procedures the author reported the detection of androgenic, estrogenic and gonadotropic substances. A comparison between the effects of the various extracts from the different sources on test animals convinced him that the testes were secreting androgenic as well as gonadotropic substances and the ovaries were secreting estrogenic substances. This report has been held by some to be the final proof for the explanation of the freemartin condition. No other publication by Atsumi, or other publications from the same laboratory, are known to the writer; the two articles in question were published in Japanese, accompanied by English abstracts. However, should it be granted that all contentions of Atsumi are correct (certainly confirmation is highly desirable) a close examination of his data reveals that effective extracts from embryonic gonad or blood were obtained from animals of 40 cm. or greater length in case of males and 60 cm. or greater in females (estimated sixth month of development); extracts from smaller, or younger, stages yielded only negative results. Data on the freemartin show that in order for the freemartin condition to develop, the effective substance would have had to be present in the blood stream and to have exercised its effects by age represented by 3.75 cm. to 15 cm. female. It is a consideration of these facts that causes the writer to hold an open mind relative to the value of this contribution in a final settlement of the problem.

Thus the question of the secretion and release of embryonic gonadal hormones in effective amounts, and the question of whether such hormones do play a role in the differentiation of the parts of the reproductive system, is in approximately a similar stage to our knowledge of other endocrine glands during development, *i.e.*, the problem remains an open one, ripe for crucial experimentation to provide a definite answer.

VII. SUMMARY

In general summary, it may be pointed out that the problem of whether individual organs of internal secretion carry on an active function and release effective hormonal substances that play a decided role in the differentiation of tissues or organs during embryonic development, is a difficult one. An appreciation of the delicacy of many normal embryologic processes, and the relative ease with which slight or more marked deviations from normal development may be brought about by minor procedures, makes more understandable the difficulties inherent in the problem. In the larval amphibians, clear answers can be given for the role of certain of the internal secretions in effecting metamorphosis with the general reconstruction of many different structures in the body; under normal conditions it is clear that the pituitary and thyroid secretions play a decided role. In the guinea pig, however, the extreme headless-type monster of Wright suggests that growth processes proceed normally up to delivery of the fetus in the absence of secretions from a pituitary body.

All the hormones ultimately exert an influence on the animal's physiologic processes (at least in the higher groups) but the determination of the period at which the influence begins to take effect is difficult. Probably in no case does the secretion from endocrine glands have a precipitous initial effect; a gradual development is characteristic, probably as regards formation of secretions within the gland, as well as in the development of sensitivity to the hormones on the part of the target organs. It is not to be understood that in this attempted analysis emphasis suggests an entire lack of effect on the part of endocrine secretions within a developing embryo, but only that the weight of evidence thus far available is insufficient to render a final answer to the general question. Emphasis is thus directed to the unsatisfactory state of the problem and to the fact that the problem deserves concerted efforts on the part of investigators to bring forth evidence that can be regarded as satisfactory.

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Association for the Study of Internal Secretions

The next Annual Meeting is scheduled at Atlantic City, New Jersey, June 8, 9 and 10, 1951, with headquarters at Haddon Hall.

American Goiter Association

The next Annual Meeting of the American Goiter Association will be held in Columbus, Ohio, May 24, 25 and 26, 1951.

VAN METER PRIZE AWARD FOR 1951

THE American Goiter Association again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Columbus, Ohio, May 24, 25, 26, 1951, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1951.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

MICHAEL REESE HOSPITAL POSTGRADUATE COURSE

MICHAEL REESE HOSPITAL POSTGRADUATE SCHOOL offers a course in Diseases of the Endocrines, Physiology and Diagnostic Methods. The class will meet from September 18 through September 29, 1950 and will be under the direction of Dr. Rachmiel Levine, Director of the Department of Metabolic and Endocrine Research. The course will consist of a balanced program of lectures and case demonstrations. Tuition is $100.00. For further information and a detailed schedule, address: Dr. Samuel Soskin, Dean, Michale Reese Hospital Postgraduate School, 29th Street and Ellis Avenue, Chicago 16, Illinois.

THE AMERICAN COLLEGE OF PHYSICIANS ANNOUNCES ITS ANNUAL SESSION AT ST. LOUIS APRIL 9–13, 1951

The American College of Physicians will conduct its 32nd Annual Session at St. Louis, Mo., April 9–13, inclusive, 1951. Dr. Ralph Kinsella of St. Louis is the General Chairman and will be responsible for local arrangements and for the program of Clinics and Panel Discussions. Dr. William S. Middleton, President of the College, Madison, Wis., will be in charge of the program of Morning Lectures and afternoon General Sessions.

Secretaries of medical societies are especially asked to note these dates and, in arranging meeting dates of their societies, to avoid conflicts with the College Meeting for obvious mutual benefits.

E. R. LOVELAND, *Executive Secretary*

P.S. It may also be noted that the 1952 Annual Session of the College will be held at Cleveland, Ohio, April 21–25, 1952.

RESEARCH FELLOWSHIPS—THE AMERICAN COLLEGE OF PHYSICIANS

The American College of Physicians announces that a limited number of Fellowships in Medicine will be available from July 1, 1951–June 30, 1952. These Fellowships are designed to provide an opportunity for research training either in the basic medical sciences or in the application of these sciences to clinical investigation. They are for the benefit of physicians who are in the early stages of their preparation for a teaching and investigative career in Internal Medicine. Assurance must be provided that the applicant will be acceptable in the laboratory or clinic of his choice and that he will be provided with the facilities necessary for the proper pursuit of his work.

The stipend will be from $2,200 to $3,200.

Application forms will be supplied on request to The American College of Physicians, 4200 Pine Street, Philadelphia 4, Pa., and must be submitted in duplicate not later than *October 1, 1950*. Announcement of awards will be made November, 1950.